A STUDY OF TWO PRIORITY POLLUTANTS, 4-CHLORO-3-METHYL PHENOL AND 2-NITROPHENOL, CONCERNING THEIR FATE AND EFFECT UPON SYSTEM PERFORMANCE IN THE BATCH ACTIVATED SLUDGE PROCESS

By

DAVID EDWARD McCARTNEY II

Bachelor of Arts

East Stroudsburg State College

East Stroudsburg, Pennsylvania

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Thesis Approved:

Don F Kincannon
Thesis Adviser

Marvin H Bates

John N Veenstra

Norman N Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

As a result of a law suit brought against the U.S. Environmental Protection Agency (EPA) by a coalition of environmentally concerned plaintiffs, the impetus has been provided to begin characterizing waste waters and effluents in terms of specific organic compounds. These environmental groups alleged that the EPA had failed to implement portions of the Federal Water Pollution Control Act (P.L. 92-500). Following a substantial research effort, the EPA responded by publishing a list of toxic pollutants for which effluent limitations would be imposed. The criteria used to select these pollutants included potential health hazard, persistence in the environment, tendency toward bioaccumulation, and evidence of synergistic propensity. Also considered were total production of the substance, use patterns, extent of likely point discharges, the consequences of exposing man or wildlife to the substances (or breakdown products), and analytical techniques available (20). This list of compounds has become known as the priority pollutants and consists of 129 individual compounds, 115 of which are organic (14). These compounds were divided into three categories. The first consisted of proven mutagens, carcinogens, and teterogens. The second group included those compounds that were chemically analogous to the first group, although not directly proved harmful. The last group included those compounds shown to be acutely toxic to biological organisms (20).

If found to be a component of a waste water, an economical treatment of the priority pollutant must exist. Strier (30) delineates the five treatment schemes under consideration by the EPA for the treatability of organic priority pollutants as being: (1) steam or air stripping, (2) oil/water separation, (3) dual media or diatomaceous earth filtration, (4) carbon adsorption, and (5) biochemical oxidation. Suggested treatment schemes for all the organic pollutants are also presented. Treatment mechanisms are based primarily on the physical and chemical properties of the compound (28, 29).

This research is confined to the study of the feasibility of employing biochemical oxidation for the treatability of two phenolic priority pollutants. This work is an outgrowth of a larger research project sponsored by the EPA to determine the compatability of priority pollutants with the treatment of municipal waste waters utilizing the activated sludge process. The emphasis here was placed on determining the fate of the priority pollutant when subjected to biochemical oxidation and any effect that the priority pollutant might have on the biological degradation of the municipal waste water components.

CHAPTER II

LITERATURE REVIEW

Of the five treatment schemes under consideration by the EPA for priority pollutant treatability, biochemical oxidation offers the potential advantages of either complete metabolism and oxidation or biochemical detoxification (conversion to a less toxic compound) of the pollutant. Forty-three of the 115 organic priority pollutants are thought to have some potential for treatment by biochemical oxidation (28). In addition, the current widespread use of biological treatment for purifying wastewaters makes a knowledge of the optimum treatment conditions for particular priority pollutants extremely important, since the need to retrofit a plant with additional, expensive unit operations might be eliminated. In a study conducted to determine the fate of priority pollutants subjected to biological treatment (7), eight of the nine organic priority compounds detected in the influent were reduced by at least 50 percent in the effluent.

Pitter (19) proposes a classification of organic compounds where degree of biodegradability and toxicity are the two basic criteria determining the behavior of any compound in the natural environment. In addition, a further subdivision of the non-biodegradable chemicals based upon their tendency to be bioaccumulated would seem reasonable from a bioenvironmental engineering standpoint. In his report on non-biodegradable and recalcitrant molecules, Alexander (1) points out that any

reasonable mechanism of recalcitrance should include these four categories of chemicals: (1) totally refractory compounds not degraded under any circumstances; (2) compounds that are degraded but only at slow rates; (3) compounds which can be degraded by microbial cultures but not natural populations, and (4) compounds which are degraded by heterogeneous populations in one or more microbial habitats but which can occasionally be quite persistent. Alexander further elaborates 15 mechanisms of recalcitrance from the following ground rules:

1. An organism must exist which can act upon the compound and it must be present at the site of the discharge. Also, the environmental conditions must be amenable to the survival of this organism.

2. If the degradatory enzymes are intracellular, the compound must penetrate the cellular membrane.

3. If the degradatory enzymes are not constitutive, they must be induced.

The two phenolic compounds selected for study were 2-nitrophenol and 4-chloro-3-methyl phenol. A summary of their physical and chemical properties as well as suggested treatment mechanisms are listed in Table 1. A crystalline solid at ambient temperature, 4-chloro-3-methyl phenol is used as an external germicide and as a preservative for glues and paints. 2-nitrophenol is used as an intermediate in various chemical and dyestuff manufacturing (29). In their listing of the frequency of occurrence of organic compounds identified in water, Shackelford et al. (24) found that 2-nitrophenol was detected six times; twice in river water and four times in chemical effluents. Another study (16) reported finding 2-nitrophenol in the effluent of a pharmaceutical plant. Shackleford et al. reported that 4-chloro-3-methyl phenol was detected

TABLE I

PHYSICAL PROPERTIES, CHEMICAL PROPERTIES, AND SUGGESTED TREATMENT MECHANISMS FOR 2-NITROPHENOL AND 4-CHLORO-3-METHYL PHENOL

Compound	Molecular Weight	Boiling Point °C	Water Solubility mg/L at 20-25 °C	Log Partition Coefficient	Suggested Treatment
2-Nitrophenol	139.1	217.2	2,100	1.8	Dual Media or Diatomaceous Earth Filter Activated Carbon Biochemical Oxidation
4-Chloro-3-Methyl Phenol	142.6	235.0	4,000	3.0	Activated Carbon Biochemical Oxidation

one time in an effluent. 2-nitrophenol is listed in the Registry of Toxic Effects of Chemical Substances (23) as having an LD_{50} on rats receiving oral dosages of 1000 to 3000 mg/kg and an LD_{60} of 100 mg/kg on dogs receiving intravenous dosages. Both 2-nitrophenol and 4-chloro-3-methyl phenol are classified as acutely toxic priority pollutants (20) and are listed as having an estimated theoretical treatability which would yield an effluent quality of 50 µg/ℓ.

Alexander (1) states that increased resistance toward degradation is imparted to disubstituted benzenes when a hydroxyl is replaced by a chloro or a nitro group. This implies that 2-nitrophenol would offer greater resistance toward biodegradation than catechol. The work of Barth and Bunch (2) verified this. However, they also reported that although nitro groups generally impart a greater resistance to biological breakdown of a particular compound, 2-nitrophenol was found to cause a microbiological oxygen demand 20 percent in excess of that required by an equal concentration of phenol. Although one study (27) observed no 5-, 10-, or 20-day BOD for 2-nitrophenol, another study (same source) reported that 22 percent of the theoretical COD was exerted by microorganisms exposed to 100 mg/L 2-nitrophenol after only 3.5 hours.

Haller (11) conducted biochemical oxidation studies with 17 monochloro, amino, and nitro benzoates and phenols. Although her work showed that the degradation of several of these compounds was affected by innoculum source and preadaptation to certain parent compounds, 2-nitrophenol was found to be taken up by the microorganisms with relative ease under all the conditions investigated. Pure culture research utilizing nitrophenols (22) brought to light a phenomenon known as cometabolism. The isolate, when exposed to paranitrophenol, was found to demand

roughly the same amount of oxygen required to degrade a similar quantity of phenol. Nitrite was produced by the isolate at a level corresponding to the molar concentration of the paranitrophenol. A transient intermediate of 4-nitrocatechol was also demonstrated. The reaction of this same isolate toward 2-nitrophenol was much different, however. Although the 2-nitrophenol concentration was reduced by the culture, no nitrite was produced and oxygen demand was less than 10 percent of that level required to degrade paranitrophenol. GC-MS data indicated that a cometabolite (probably nitrohydroquinone) was present at the same concentration as that of the original substrate. This cometabolite was thought to be a detoxification product of 2-nitrophenol.

In another study (25), two cultures which could metabolize nitrophenols were isolated from a trickling filter innoculum. One strain could metabolize 2-nitrophenol while the other utilized paranitrophenol. Neither strain could metabolize the other nitrophenolic isomer. As 2-nitrophenol was degraded, nitrite was produced. It was theorized that 2-nitrophenol was converted through catechol to cis-cis muconic and/or ß-ketoadipic acids, then on to more ubiquitous metabolic pathways. Based upon limited data accumulated from an existing publicly owned treatment facility, 2-nitrophenol was not found in the influent (six samples) or sludges (seven samples), but was detected three (eight samples) times in the effluent (7).

Very little information was found which pertained specifically to 4-chloro-3-methyl phenol (p-chloro-m-cresol). Alexander (1) does state that monochlorophenols with the halogen ortho or para to the hydroxyl offer less degradatory resistance than when the halogen is in the meta position. Relative to phenol, cresols were found to produce a greater

or equivalent demand for oxygen according to Barth and Bunch (2). This same study indicated that chloro substitution of cresols reduced the oxygen demand. Pitter (19) found that acclimated cells removed 2-nitrophenol at a rate less than 20 percent that of phenol removal. Here, COD was reduced by 97 percent. It was reported that an oxygen uptake value equivalent to 59 percent of the theoretical chemical oxygen demand by microorganisms acting upon 4-chloro-3-methyl phenol (26). Pitter (19) found that ortho, meta, and para cresol were removed at a rate of about 70 percent that of phenol by acclimated cultures. Ninety-five percent COD reduction was reported. Limited data gathered from a publicly owned treatment facility indicated that 4-chloro-3-methyl phenol was present in 5 percent (one sample) of the influents tested, but was not detected in the effluent or sludge samples analyzed (7).

Several researchers have conducted work involving multicomponent substrate wastewaters. Gaudy, Gaudy, and Kolmorit (9) demonstrated sequential substrate removal in both pure culture and heterogeneous batch microbial systems utilizing glucose and sorbitol as substrates. Manickam (18), in his research dealing with multi-substrate wastewaters and qualitative shock loading of continuous flow activated sludge systems, noted that changes in the biokinetic constants occurred when qualitative shock loads from glucose to a mixture of glucose and sorbitol to sorbitol were administered. It was suggested that these variations in the biokinetic constants may have been caused by predominance changes and variability in the microbial components of the heterogeneous populations. Although COD "leakage" was generally noted at the onset of the shocks (later abating), the components of this "leakage" were rarely the original substrates. Su (31) worked extensively with batch and continuous multi-

component substrate systems. Certain substrates, among them sorbitol, galactose, glycerol, and xylose, were found to exhibit sequential substrate removal in batch systems when glucose was also present. Glucose almost always was the preferred substrate. This work also emphasized the fact that a residual COD remained that was not composed of any of the initial substrates. Chian et al. (4) also reported sequential substrate removal in a batch system receiving a naturally occurring wastewater (landfill leachate). Again, the formation of intermediates was noted and an attempt was made to identify them. Decomposition was found to occur in three stages with the carbohydrates being metabolized first, fatty acids second, and the phenolics as well as the intermediates found during the first two phases were removed last.

An area of great concern regarding the biological treatment of priority pollutants is the potential for microbiological toxicity. Research involved with phenol (5) indicated that a phenol concentration of 2000 mg/L was bacteriacidal but that lower concentrations could be degraded. Other research utilizing pentachlorophenol (12) and glucose batch systems demonstrated that for unacclimated cells, shock load pentachlorophenol (PCP) concentrations of 5, 15, and 30 mg/L impaired the uptake of glucose. Uncoupling of oxidative phosphorylation and dispersing of the floc were evident. Cells acclimated to PCP were thought to have undergone a predominance change due to pronounced change in color of the microorganisms. After seven-hour substrate removal tests, phenol analyses indicated no PCP removal. Other research (10) seemed to imply that PCP was removed by batch cultured organisms after prolonged acclimation and stepwise increase in PCP concentration (inferred from COD data). Parameters suggested as means by which to measure toxicity are

oxygen uptake, sludge dehydrogenase activity, and ATP content (17). Broeker and Zahn (3) compared results from five toxicity test procedures to the actual performance of a pilot activated sludge plant exposed to 3,5-dichlorophenol. All five tests (2- and 20-hour oxygen uptakes, dehydrogenase activity, gas formation in fermentation tubes, and inhibition of cell division in Pseudomonas) all predicted observed toxicity threshold levels of the pilot plant toward 3,5-dichlorophenol rather well. An interesting observation pertaining to this study was that the activated sludge could assimilate a shock load after an acclimation period but without the presence of 3,5-dichlorophenol, the sludge would lose its adaptive capacity in three or four days. Unfortunately, no mention was made of sludge age in the pilot plant in that it would be of some interest to compare the time required to lose its adaptive capacity to various sludge retention times. It was also noted that effluent quality was impaired to a lesser degree upon shock loading at low sludge loadings.

Although not the focus of this work, some mention should be made regarding air stripping of volatile compounds in that, as a result of sludge aeration, there is a potential for volatile compounds to be removed by nonbiological processes. Thirty-six of the priority pollutants are listed as being amenable to steam or air stripping (28). Gaudy and Engelbrecht (8) found that the critical parameters affecting the overall transfer coefficient for stripping were temperature, unit air flow, and tank geometry. One case was documented where an aldehyde was chemically converted to another compound (most likely an acid) which gave rise to a situation whereby specific compound analyses pointed toward a stripping phenomenon but a more nonspecific test of organic content (COD) did not.

Although employed in a few full scale applications (13), batch systems are not generally utilized as a unit operation in wastewater treatment systems. Rather, completely mixed or plug flow activated sludge units are much more common in the field. Although purported to simulate a batch reactor as the influent moves as a slug through the reactor, plug flow units have been reported to more closely resemble a completely mixed system (15). Batch units are, therefore, most often used to indicate whether or not a particular waste can be biochemically oxidized and to approximate the response of a continuous flow reactor. However, there is some question pertaining to the validity of employing data derived from batch units to predict the performance of a continuous flow reactor (10).

CHAPTER III

MATERIALS AND METHODS

3.1 General Research Approach

Batch-fed (fill and draw) activated sludge units, as shown in Figure 1, were employed in these studies. The cylindrical, 3.75 inch diameter, 3.5 liter capacity reactors were supplied with sufficient diffused air (2L/min) to maintain greater than 2 mg/L dissolved oxygen even after feeding. Initially, seeding organisms were obtained from the Stillwater Municipal Sewage Treatment Plant's (SMSTP) primary clarifier effluent and supplied with a 200 mg/L glucose and sewage feed until an adequate supply of microorganisms developed.

Standard operating procedure for the batch activated sludge units follows:

1. One liter of mixed liquor was removed from the reactors which contained 3 liters of activated sludge which had 23 hours to metabolize the previous day's feed.

2. The diffused air supply was removed from the reactors and one hour was allowed for settling.

3. One liter of settled supernatant was withdrawn from each unit.

4. To two liters of primary effluent (obtained daily from the SMSTP), 300 mg/L glucose and 150 mg/L ammonium sulfate were added. It was determined that sufficient phosphorus was present in the domestic sewage. This mixture was then mixed with the one liter of concentrated

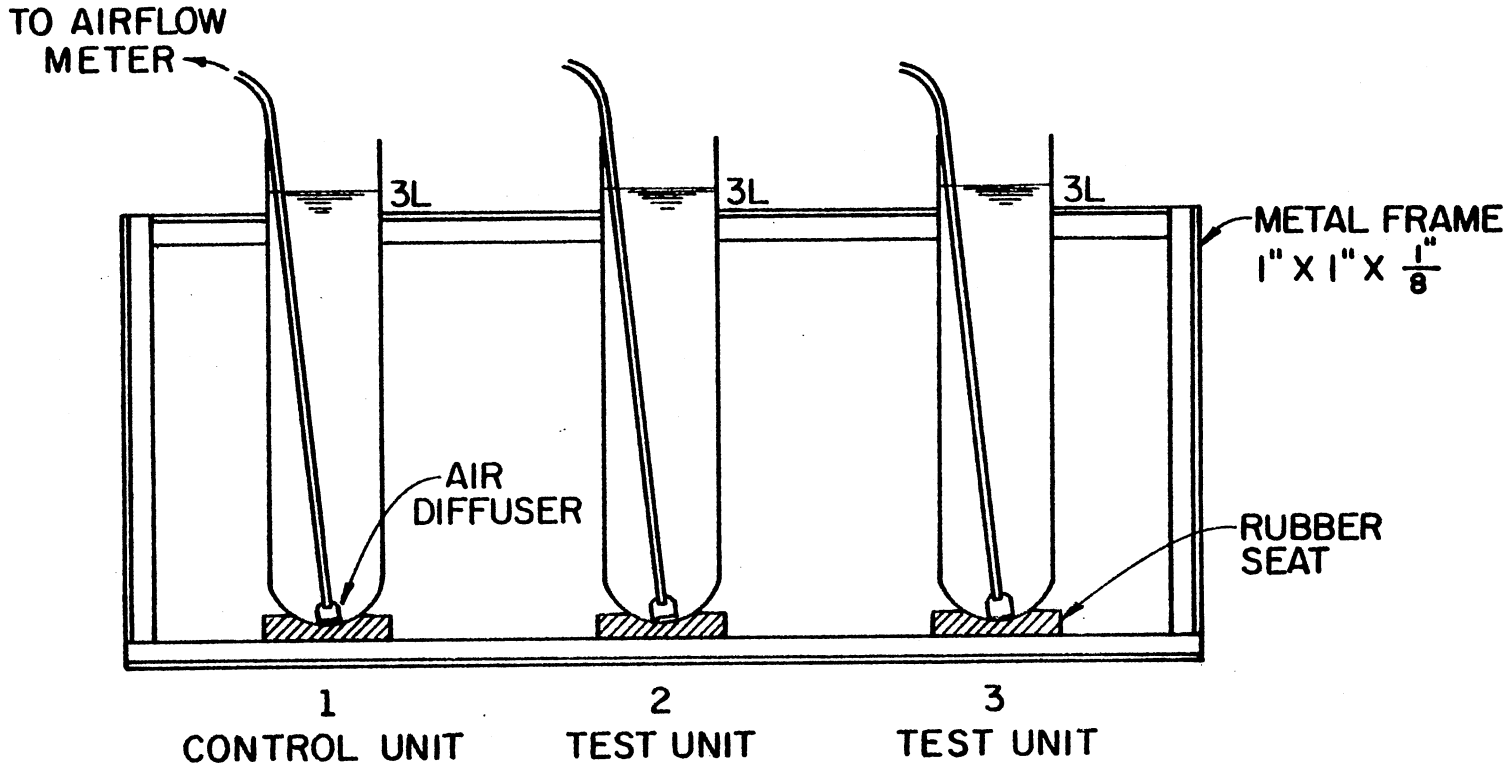


Figure 1. Illustration of the Batch Activated Sludge Reactor

sludge remaining in the unit. The air diffuser was then replaced. After 23 hours the procedure was repeated.

Parameters monitored regularly were pH and temperature as well as suspended solids and soluble chemical oxygen demand (COD) both after the 23-hour metabolic period (before feeding) and immediately after introducing the wastewater (after feeding). Parameters monitored intermittently were soluble total organic carbon (TOC), gas chromatographic or colorometric analyses for priority pollutants, settled supernatant suspended solids, mixed liquor, dissolved oxygen uptake rate, and soluble carbohydrate. When an analysis is designated as soluble, the sample was passed through a millipore filter (0.45 μm).

3.2 Determination of the Compatability of the Priority Pollutant With the Biological Treatment of a Glucose-Sewage Wastewater

In this work two priority pollutants, 2-nitrophenol (2NP) and 4-chloro-3-methyl phenol (CMP), were selected for study. Two batch units were operated under identical conditions for a brief control period (one to two weeks). After this control period, one of the units (the test unit) began receiving 5 mg/L of the priority pollutant under investigation in its feed. The other unit, termed the control, never received the priority pollutant but, in all other respects, was identical to the test unit. Running these two units simultaneously permitted a more meaningful assessment of the effect of the priority pollutant on the batch system. After allowing a reasonable period of time to elapse in order to determine the effect of the priority pollutant on the batch system

performance (three to five weeks), the dosage was increased to 25 mg/L priority pollutant. The effect of the priority was again determined. The priority pollutant concentration was finally increased to 50 mg/L and its effect measured. Following the 50 mg/L priority pollutant concentration, a series of cyclic dosages were administered to determine if removal of the compound from the feed for several days followed by reintroduction of the priority pollutant would have any adverse effect on the batch system operation. Evaluation of this long-term data with special reference to 23-hour supernatant soluble COD, soluble TOC, and suspended solids was expected to provide valuable insight regarding the compatability of the priority pollutant with the activated sludge process. Slowing the rate of substrate removal was also thought to be a potential problem that a priority pollutant might introduce. For this reason substrate removal tests were performed at least once during each priority pollutant concentration. During these studies, suspended solids concentrations, soluble COD and often soluble TOC, priority pollutant concentration, soluble carbohydrate, and dissolved oxygen uptake rate were periodically monitored during the 23-hour metabolic period.

3.3 Determination of the Fate of the Priority Pollutant During the Batch Biological Treatment Process

The long-term data and the substrate removal data, especially the 2NP and CMP analyses, provided some information relating to the fate of the priority pollutant in the batch systems. Air stripping tests were also performed in the same reactor type and under the same conditions of air flow and temperature as the biological units. This was done to

determine whether the removal of the compound was due to the biomass or to the air stripping capability of the diffused-air-supplied batch reactor. In addition, independent substrate removal tests utilizing the priority pollutant as the sole organic carbon source and employing a seed culture obtained from the long-term acclimated batch systems were run. Substrate removal, total suspended solids, suspended carbohydrate, suspended protein, and dissolved oxygen uptake rate provided excellent information pertaining to the fate of the priority pollutant in biological systems.

A summary of all of the analytical techniques used for these investigations is found in Table II.

TABLE II

ANALYTICAL TECHNIQUES EMPLOYED IN THESE INVESTIGATIONS

Suspended Solids	Membrane Filter Technique, Milipore Type H.A.--0.45 μm	Standard Methods (26) Procedure substituting a membrane filter for a glass fiber
COD	Chromic Acid Oxidation	Standard Methods (26)
TOC	Beckman Model 915 TOC Analyzer	Beckman Instruments
2NP and CMP	F&M Model 810L-12 Gas Chromatograph; Extraction	U.S. EPA Procedure (6)
2NP Colorimetric	Add NH_4OH:NH_4Cl Buffer pH 8.0 and compare with known standards on Spec 20; wavelength 400	Developed In-House
pH	Orion Research Model 701 pH meter	
Dissolved Oxygen Uptake Rate	Weston & Stack Model 330 D.O. Analyzer in a BOD bottle mixed with a magnetic mixer. Cumulative value derived from an integration of the uptake rates	
Suspended Protein	Biuret Technique	OSU M-2 Manual (21)
Suspended Carbohydrate	Anthrone Technique	OSU M-2 Manual (21)
Soluble Carbohydrate	Anthrone Technique	OSU M-2 Manual (21)

CHAPTER IV

RESULTS

4.1 4-Chloro-3-Methyl Phenol

4.1.1 Long Term Data

A difficulty encountered when attempting to compare analytical results of biological systems is that a certain amount of variation would be expected even between systems that were operated identically. An effort was made to separate this inherent variation from statistically significant discrepancies which may have been induced by the priority pollutant. This was accomplished by developing probability plots of variations recorded between three control units operated identically for a period of 140 days (which included 60 sampling days from each unit). A base period of comparison of 10 sample days was selected. Variations between Number 1 and Number 2 control units were ranked for each of six periods of comparison. The same procedure was followed for comparisons of control units Numbers 1 and 3, as well as for control units Numbers 2 and 3. If the value for the first control was greater than the control to which it was being compared, a positive variation was assigned. If the reverse was true, the variation was considered to be negative. These comparisons were then composited. A mean value and standard deviation for each rank were determined and plotted on arithmatic probability paper. Parameters considered for statistical analyses were soluble 24-hour COD, 24-hour suspended solids and solids yield. An example of these probability plots is presented in Figure 2. Using the mean probability line, it

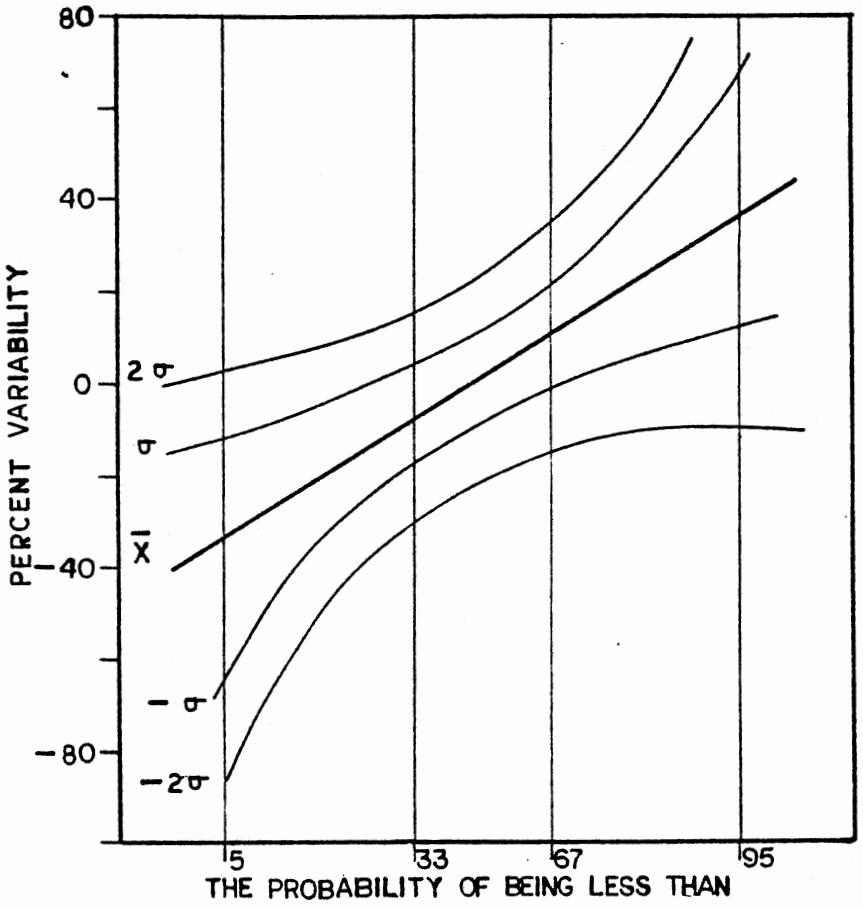


Figure 2. Inherent Variability Expected Between Three Similarly Operated Batch Activated Sludge Reactors for Ten Consecutive Sampling Days.

it can be seen that 70 percent of the time the variability observed in soluble 24-hour COD between two identically operated control units would be less than 13 percent. Thirty percent of the time, one would expect this variability to be less than -10 percent (show high variability in the negative direction). When comparing test unit performance with that of the control, a probability plot was determined and superimposed onto the control plot in order to judge if there were any statistically significant discrepancies (Figures 4, 5, 6, 13, 14, 15).

It should be noted that solids yield values were simply calculated as the increase in suspended solids concentration after 24 hours divided by the soluble COD removed. Suspended solids concentrations of the domestic primary clarified waste water used in feed preparation generally averaged 50 mg/L and could range from 20 to 100 mg/L. The chemical composition of these solids could quite conceivably be variable, also. Probably due to day-to-day variation in feed suspended solids concentration and composition, yield calculations showed tremendous fluctuations. This experimental condition could also explain the spiked shape of the suspended solids curves found in the long-term batch system plots.

4.1.2 Control Period

Figure 3 illustrates four months of suspended solids and soluble substrate data for both the control and test units. Table III presents statistical analyses of these same data. Each dosing period was separated into two parts. This was done in order to determine if the compound caused either an initial reaction that was later mitigated or had a delayed effect. At the conclusion of a brief control period, it can be seen that both units exhibited similar performance with respect to solids production and COD removal. Figures 4, 5, and 6 show probability plots of the variability observed between the control and test units for 24-hour soluble

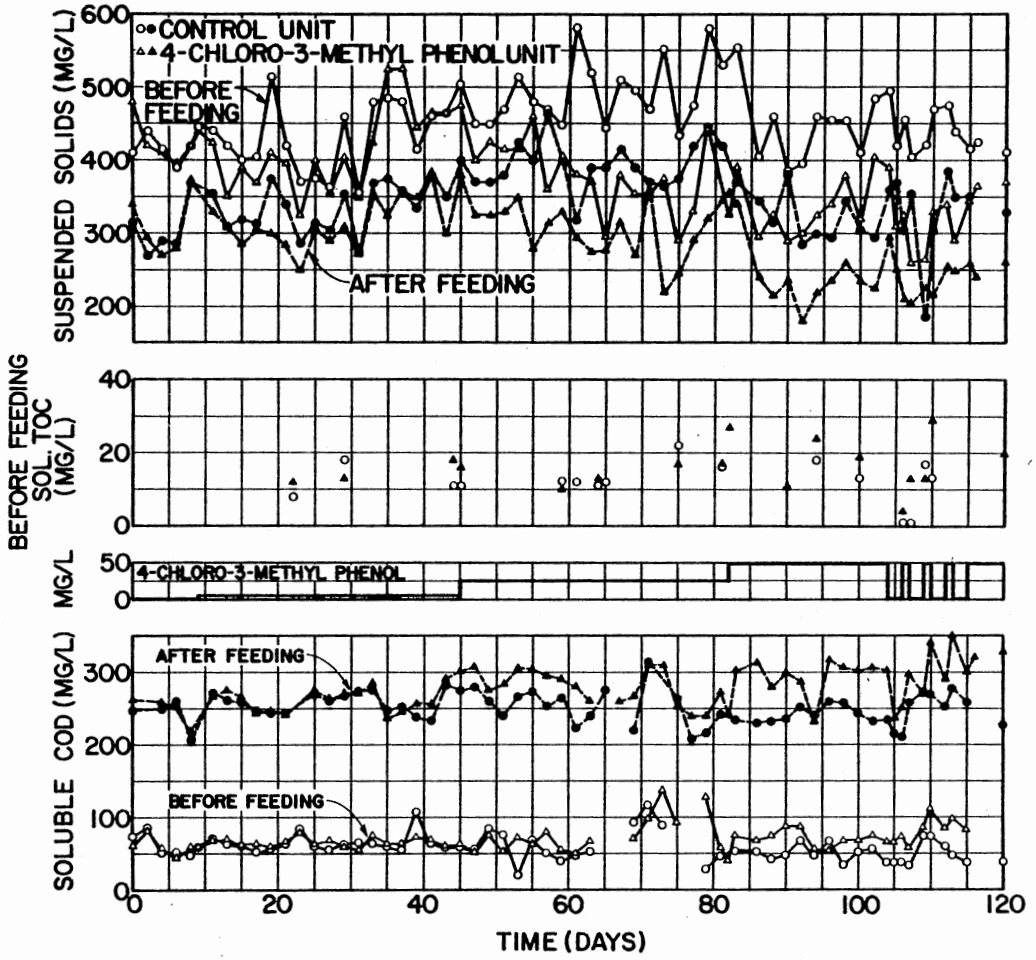


Figure 3. Long-Term Suspended Solids and Soluble Substrate Concentrations for a Batch Control Unit and a Test Unit Exposed to Increasing Concentrations of 4-Chloro-3-Methyl Phenol

TABLE III

LONG TERM STATISTICAL ANALYSIS FOR A BATCH UNIT SUBJECTED TO VARYING DEGREES OF 4-CHLORO-3-METHYL PHENOL

Operating Days	CMP Dosage mg/L		Control Initial sol.COD mg/L	Control 24 hr sol.COD mg/L	Control 24 hr SS mg/L	Control Initial SS mg/L	Control Solids Yield	4-Chloro-3-Methyl Phenol Initial sol.COD mg/L	4-Chloro-3-Methyl Phenol 24 hr sol.COD mg/L	4-Chloro-3-Methyl Phenol 24 hr SS mg/L	4-Chloro-3-Methyl Phenol Initial SS mg/L	4-Chloro-3-Methyl Phenol Solids Yield
1-8	0	n	4	5	5	5	4	4	5	5	5	4
		$\bar{x}$	240	62	415	306	.50	248	61	425	311	.56
		σ	24	17	18	39	.13	20	14	32	43	.18
9-24	5	n	6	8	7	7	5	6	8	8	7	6
		$\bar{x}$	254	64	414	329	.46	258	67	388	295	.46
		σ	11	9.9	26	30	.06	14	6.7	38	25	.16
25-44	5	n	10	10	10	10	10	10	10	10	10	10
		$\bar{x}$	260	65	434	342	.52	265	66	436	325	.56
		σ	16	15	52	34	.19	18	6.0	61	35	.28
45-60	25	n	8	8	8	8	8	8	8	8	8	8
		$\bar{x}$	264	57	473	401	.33	295	64	419	329	.39
		σ	13	20	25	32	.22	11	11	36	28	.17
61-81	25	n	9	7	11	11	5	10	10	10	11	8
		$\bar{x}$	245	68	509	391	.66	269	81	357	283	.49
		σ	35	32	50	35	.13	25	32	45	40	.36
82-92	50	n	5	5	5	5	5	6	6	6	6	6
		$\bar{x}$	237	54	440	339	.55	288	72	321	228	.43
		σ	8.8	9.9	71	39	.38	25	18	37	27	.19
93-104	50	n	6	6	6	6	6	6	6	6	6	6
		$\bar{x}$	244	49	460	317	.75	295	64	360	245	.50
		σ	12	12	30	28	.22	31	7.8	36	28	.16
105-120	50-0-0	n	4	4	4	4	4	4	4	4	4	4
		$\bar{x}$	249	52	441	306	.70	279	82	319	237	.42
		σ	27	18	29	87	.40	22	5.7	37	24	.18
105-120	0-0-50	n	3	3	3	3	3	4	3	4	4	3
		$\bar{x}$	270	52	438	337	.48	328	89	311	227	.30
		σ	10	20	33	28	.32	24	28	46	21	.18

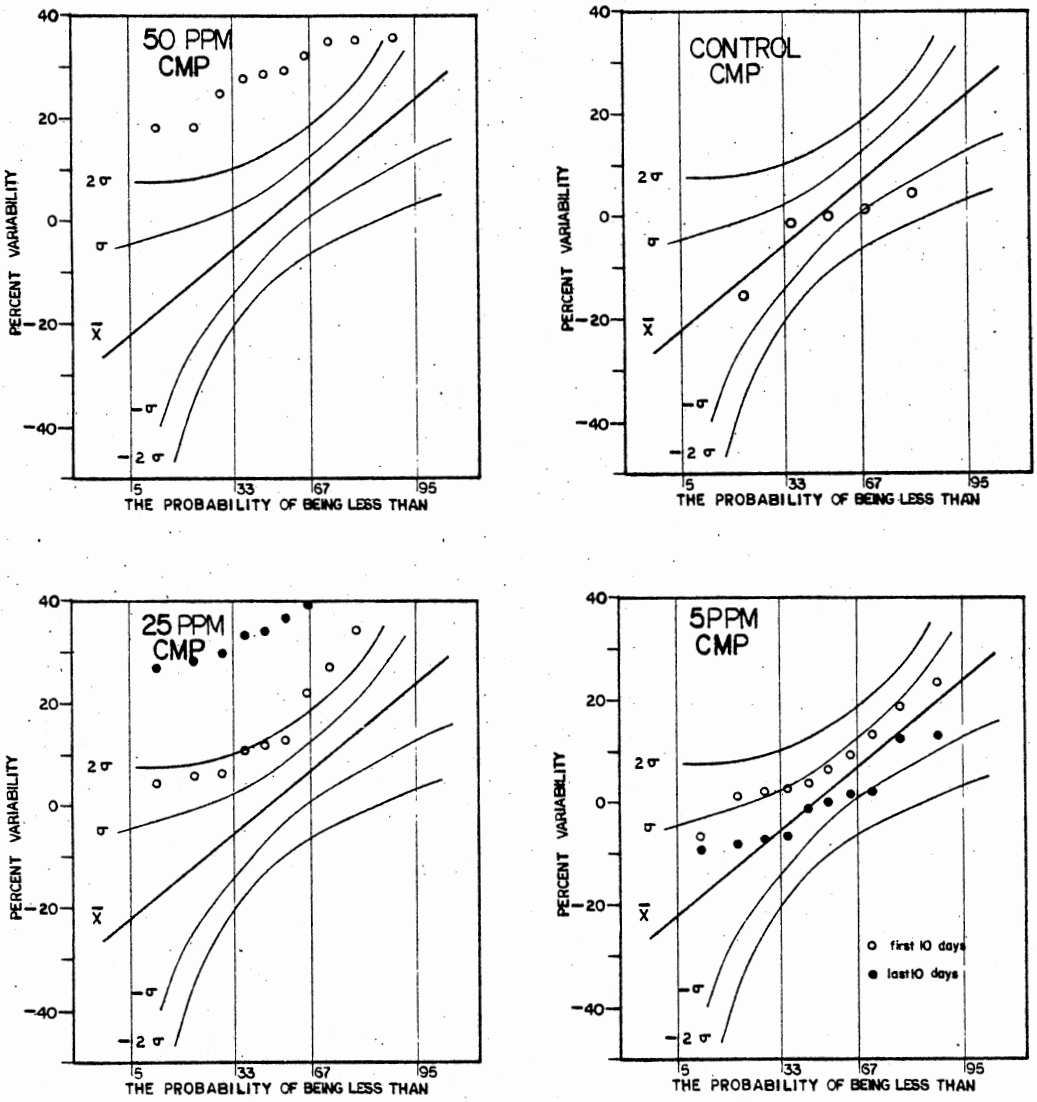


Figure 4. Analysis of Statistically Significant Effects of CMP Upon 24-Hour Suspended Solids in the Batch Unit

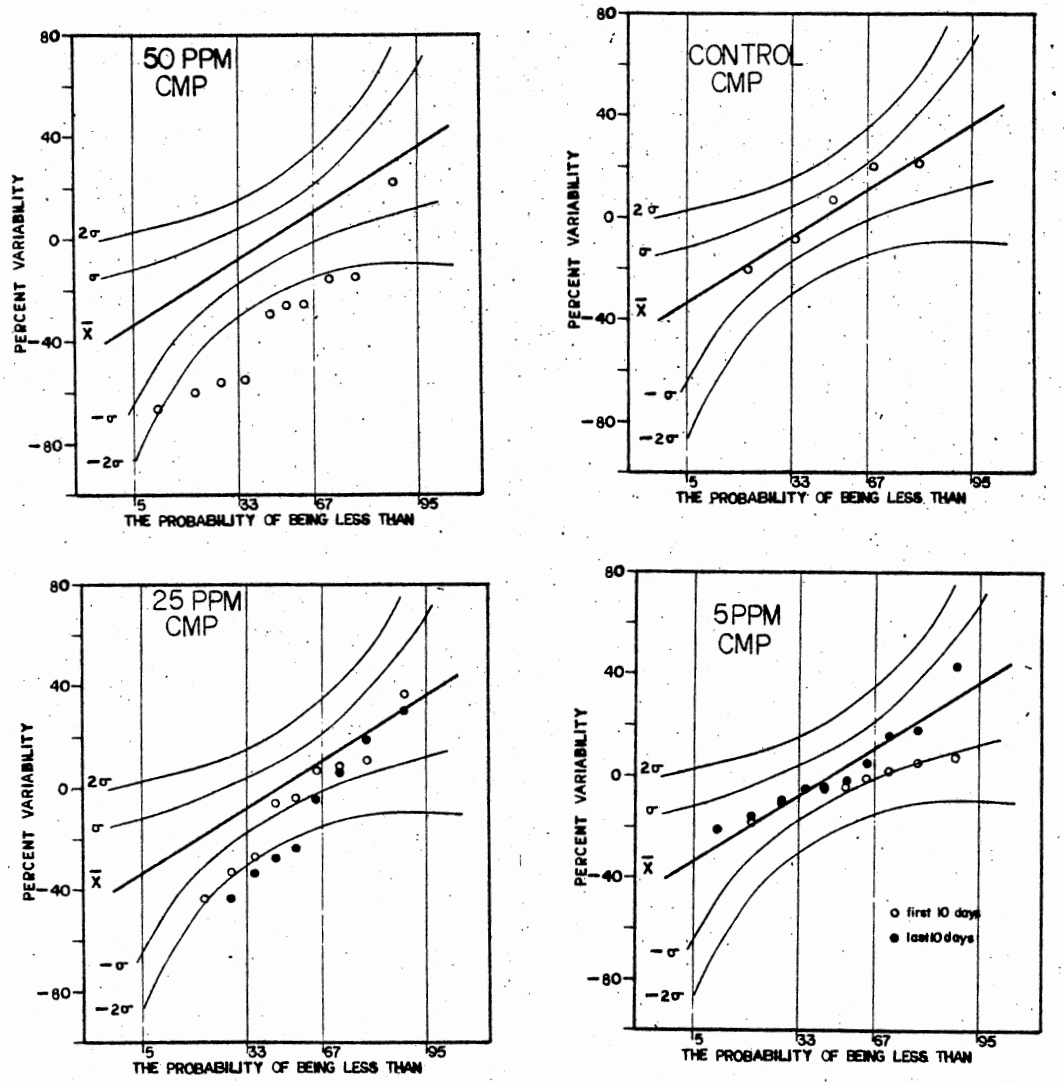


Figure 5. Analysis of Statistically Significant Effects of Various Dosages of CMP Upon Soluble 24-Hour COD in the Batch Unit

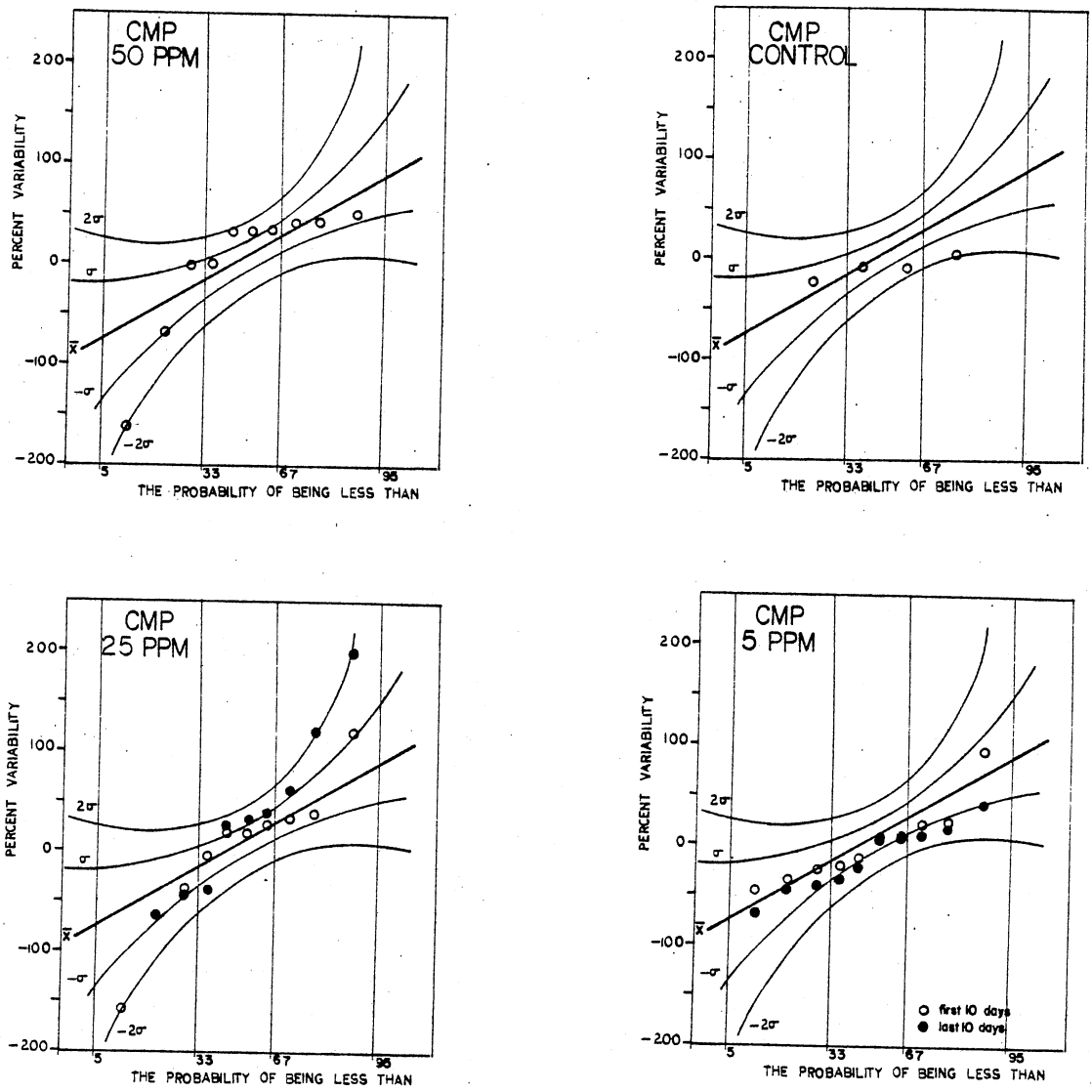


Figure 6. Analysis of Statistically Significant Effects of Various Dosages of CMP Upon Solids Yield in the Batch Unit

COD, 24-hour suspended solids and solids yield, respectively. Positive variability indicates that the control unit value was higher. It can be seen that variations observed during the control period in all three were generally within one standard deviation of the control comparison. Unfortunately, the number of sample comparisons for this control period is less than 10, and a strict statistical relation to the 10-day comparison period will not be accurate.

4.1.3 5 mg/L CMP Dosage

After addition of 5 mg/L CMP to the test unit feed, a slight decrease in suspended solids was noted during the first 16 days of this dosage (Figure 3).

Statistical evaluation of the data (Figures 4, 5, and 6) shows that nearly all the variability lies within one standard deviation of the inherent variability expected between control units, but that a weak trend of higher 24-hour soluble COD and lower 24-hour suspended solids of the test unit relative to the control unit is present. The remaining 20 days during which 5 mg/L CMP was fed to the test unit indicated that the variability in 24-hour suspended solids and soluble COD between the test and control units exhibited no statistically significant differences. Solids yield demonstrated a weak trend of the test unit producing a higher solids yield than the control. Soluble TOC analyses exhibited no consistent trend. Gas Chromatography (GC) analyses determined after 33 days of acclimation to 5 mg/L CMP indicated that no detectable levels of CMP were found in the 24-hour settled supernatant or the sludge. It should be noted that after approximately 29 days of exposure to 5 mg/L CMP, the test unit sludge was observed to have changed to a dark brown as opposed to the golden brown color which characterized the control sludge. Further, at the same time, the 24-hour pH of the test unit became consistently higher than that

of the control and remained as such for the duration of the study (Table IV).

4.1.4 25 mg/L CMP Dosage

Increasing the CMP concentration in the test unit from 5 mg/L to 25 mg/L produced an effect that was obvious on both the long term plot (Figure 3) and the probability plots pertaining to this period (Figure 4, 5, and 6). Statistically, a trend of decreasing suspended solids in the test unit relative to the control was observed during the first 20 days of application of 25 mg/L CMP. All the variations were greater than one standard deviation from that which could be expected from two control units. In fact, half of the variation was greater than two standard deviations from expected control variation indicating almost certainly that the CMP dosage was the cause for the decreased suspended solids concentration of the test unit. 24-hour soluble COD exhibited a weak statistical trend of being higher in the test unit. Although most of the data variability was within one standard deviation of the inherent control variation, a significant amount of the data was found to be outside the limit of one negative standard deviation. Solids yield statistical comparison demonstrated little difference between the inherent control variation and that observed between the test unit and control. Limited data concerning residual TOC indicated little difference between the test unit and the control.

The last 20 days of administration of the 25 mg/L CMP dosage resulted in a continuing of the trends that occurred during the first 20 days of 25 mg/L CMP with respect to 24-hour soluble COD and suspended solids. Based upon mean values, 24-hour suspended solids concentrations of the

TABLE IV

pH DETERMINATIONS FOR CONTROL AND 4-CHLORO-3-METHYL PHENOL BATCH UNITS

	4-Chloro-3-Methyl Phenol Dosage mg/L	Control Unit pH	Test Unit pH
n	0	5	5
$\bar{x}$		8.4	8.4
σ		0.13	0.16
n	5	18	18
$\bar{x}$		7.8	7.9
σ		0.42	0.44
n	25	17	17
$\bar{x}$		7.7	8.3
σ		0.21	0.18
n	50	11	11
$\bar{x}$		7.8	8.4
σ		0.32	0.07
n	0-0-50	4	4
$\bar{x}$		7.9	8.4
n	50-0-0	3	3
$\bar{x}$		7.9	8.3

test unit were 30 percent lower than those of control, while soluble COD values of the test unit exceeded those of the control by 19 percent. Statistically, those trends became more significant. Solids yield, on the other hand, showed many points which were greater than one standard deviation from the variation inherent in the control units themselves, but the direction of the variation was random. However, significant differences between control and test unit residual TOC were not found. In regard to GC analyses for CMP, it was found that upon first increasing the CMP dosage from 5 mg/L to 25 mg/L, 33 percent of the initial CMP present was found to have remained in the unit after 24 hours. Subsequent analyses after 16, 41, and 42 days showed no detectable levels of CMP in the 24-hour supernatants representing removal efficiencies of at least 89 percent.

4.1.5 50 mg/L CMP Dosage

When subjected to an influent CMP concentration of 50 mg/L, 24-hour suspended solids concentrations of the test unit were 24 percent lower than those of the control while test unit 24-hour soluble COD exceeded control values by 33 percent (based upon mean values). Figures 4 and 5 illustrate that these differences are statistically significant with most of the variations greater than two standard deviations from the inherent variability expected within control units. No consistent, statistically significant differences were noted between test unit and control solids yield at this dosage.

Residual TOC showed a trend of being higher in the test unit. After 22 days of acclimation to the 50 mg/L CMP feed dose, GC analyses of the 24-hour supernatant yielded no detectable levels of CMP corresponding to at least 98 percent removal efficiency.

4.1.6 Cyclic Dosages of 50 mg/L CMP

The final twelve days of the study consisted of cyclic dosages of 50 mg/L CMP which was administered on every third day while no CMP was added on the other two days. The data indicated that significantly higher residual COD and TOC values were recorded on those days following 50 mg/L CMP dosages when compared to those days when no CMP was added. Within an accuracy range of two to three percent, solids yield and suspended solids concentrations of the test unit showed no marked differences whether or not CMP was added. It should also be noted that the pH of the test unit remained approximately the same magnitude higher over the control pH whether CMP was included in the feed or not. However, a more reddish color appeared in the test unit on those days when CMP was added. This red component was found to be a soluble substance since filtered samples absorbed light at 540 μm.

4.1.7 GC Analyses

Table V presents a summary of the GC data collected for CMP as well as the calculated feed concentrations that should have resulted. The CMP dosed at 50 mg/L was calculated on the basis of the three liter reactor volume while the other CMP dosages were calculated based on the two liters of feed added.

4.1.8 Supernatant Suspended Solids

Table VI summarizes the 24-hour settled supernatant suspended solids content of both the control and the test unit in an effort to give some indication of the solids-liquid separation tendency. It appeared that

TABLE V

GC ANALYSES FOR 4-CHLORO-3-METHYL PHENOL

Description	Calculated Feed Conc. (mg/L)	Measured Feed Conc. (mg/L)	Measured 24-hr Supernatant Conc. (mg/L)
After 33 days of acclimation to 5 mg/L CMP	3.33	7.1	<1.6
First day of 25 mg/L CMP	16.70	25.2	8.6
After 16 days of acclimation to 25 mg/L CMP	16.70	17.6	<2.0
After 41 days of acclimation to 25 mg/L CMP	16.70	---	<0.8
After 42 days of acclimation to 25 mg/L CMP	16.70	18.8	<1.3
After 38 days of acclimation to 50 mg/L CMP	50.00	48.5	<0.8

Extracted sludge during 5 mg/L CMP dosing period had no detectable CMP (<1.6 mg/L).

TABLE VI

4-CHLORO-3-METHYL PHENOL BATCH UNIT 24-HOUR SUPERNATANT SUSPENDED SOLIDS SETTLED FOR ONE HOUR

Days	CMP Dosage mg/L	Control SS mg/L	Test Unit SS mg/L
8	0	32	30
9	5	47	53
15	5	19	46
22	5	3	27
n		3	3
$\bar{x}$		23	42
29	5	14	33
37	5	22	24
44	5	24	26
n		3	3
$\bar{x}$		20	28
45	25	10	32
51	25	32	35
59	25	32	77
n		3	3
$\bar{x}$		24	48
65	25	33	16
75	25	22	20
81	25	31	35
n		3	3
$\bar{x}$		29	24
94	50	41	31
100	50	26	14
n		2	2
$\bar{x}$		33	22
106	50→0→0	19	27
109	50→0→0	17	18
112	50→0→0	15	23
115	50→0→0	14	23
n		4	4
$\bar{x}$		16	23
107	0→0→50	30	37
113	0→0→50	20	33
116	0→0→50	21	25
n		3	3
$\bar{x}$		24	32

after first initiating a 5 mg/L CMP dosage in the test unit, 24-hour supernatant suspended solids (settled one hour) were significantly higher than those of the control. However, at the end of the 5 mg/L dosing period, the differences in 24-hour supernatant suspended solids were greatly reduced. The same trend was observed when the CMP dose was increased to 25 mg/L. At first, 24-hour supernatant suspended solids were markedly higher in the test unit but after approximately 20 days of acclimation, these differences were mediated. During the period when 50 mg/L CMP was included in the test unit feed, one of the two analyses performed indicated that the test unit supernatant suspended solids were actually lower than those of the control. During the cyclic addition of 50 mg/L CMP, the test unit suspended solids concentrations were 25 percent to 30 percent higher than those of the control. However, no significant differences in test unit suspended solids of the supernatants were observed between analyses performed when 50 mg/L CMP was administered and when no CMP was included in the feed.

Periodic microscopic examinations of the sludges of both units failed to show any major differences in flocculation or protozoan species and numbers. Filamentous organisms were never present in noticeable quantities.

4.2 Substrate Removal Tests—4-Chloro-3-Methyl Phenol

4.2.1 5 mg/L CMP

Figure 7 illustrates the response of the batch fed unit receiving 200 mg/L glucose, municipal wastewater (primary clarifier effluent), and a shock load of 5 mg/L CMP compared to that of a control which received

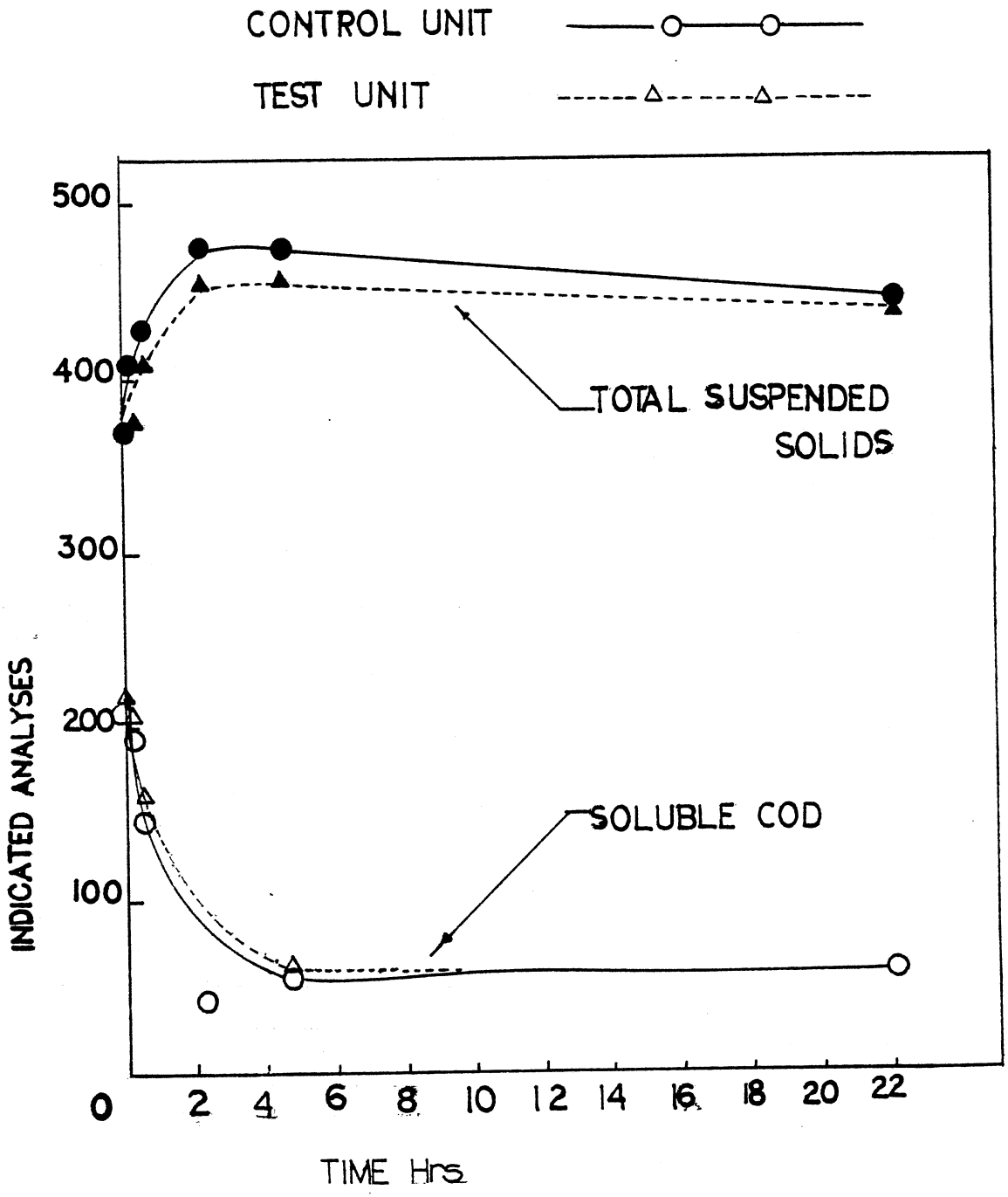


Figure 7. Substrate Removal Test; 0-5 ppm CMP; 22°C

no CMP. Substrate (COD) removal rates and efficiencies were nearly identical but a slight decrease in the 22 hour suspended solids concentration, as well as the .5, 2.2, and 4.7 hour solids determinations was noted relative to the control.

4.2.2 25 mg/L CMP

Figure 8 depicts the response of the test unit after increasing the CMP dosage from 5 mg/L to 25 mg/L. The control unit, which had never been exposed to CMP, received a feed consisting of only 300 mg/L glucose plus primary clarifier effluent. Its substrate removal and growth characteristics are plotted also. GC analyses indicated that approximately one-third of the initial CMP administered remained in the test unit after 24 hours. With the exception of the 24-hour COD reported for the test unit, the general trend of the TOC and COD data is that any increase of test unit substrate concentration above the control unit values could be attributed to the CMP still remaining in the unit. Throughout the run, test unit suspended solids were less than those of the control unit.

Figure 9 illustrates the performance of the test unit after 36 days of acclimation to 25 mg/L CMP. By this time it had become obvious that the test unit and control unit sludges were considerably different (as determined by color, pH and suspended solids concentration). Therefore, for this and subsequent substrate removal tests performed using CMP, the control sludge was obtained from the long term test unit and simply not dosed with CMP. Although the 22-hour suspended solids concentration of the test unit was quite similar to that of the control, the test unit exhibited higher suspended solids concentrations from the sixth to the sixteenth hour of the run. Soluble COD and TOC of the test unit showed a

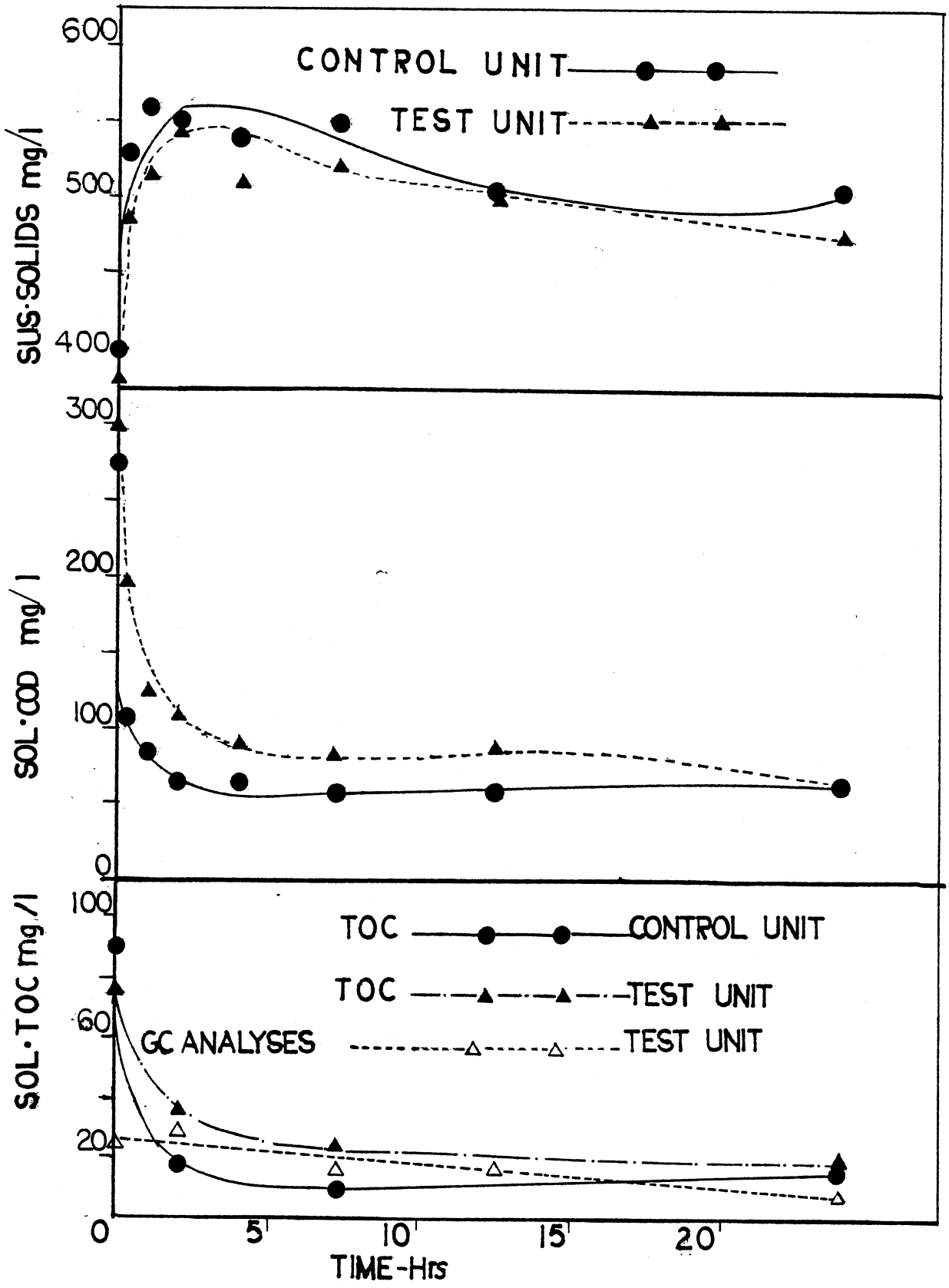


Figure 8. Substrate Removal Test; 5-25 ppm CMP; 23°C

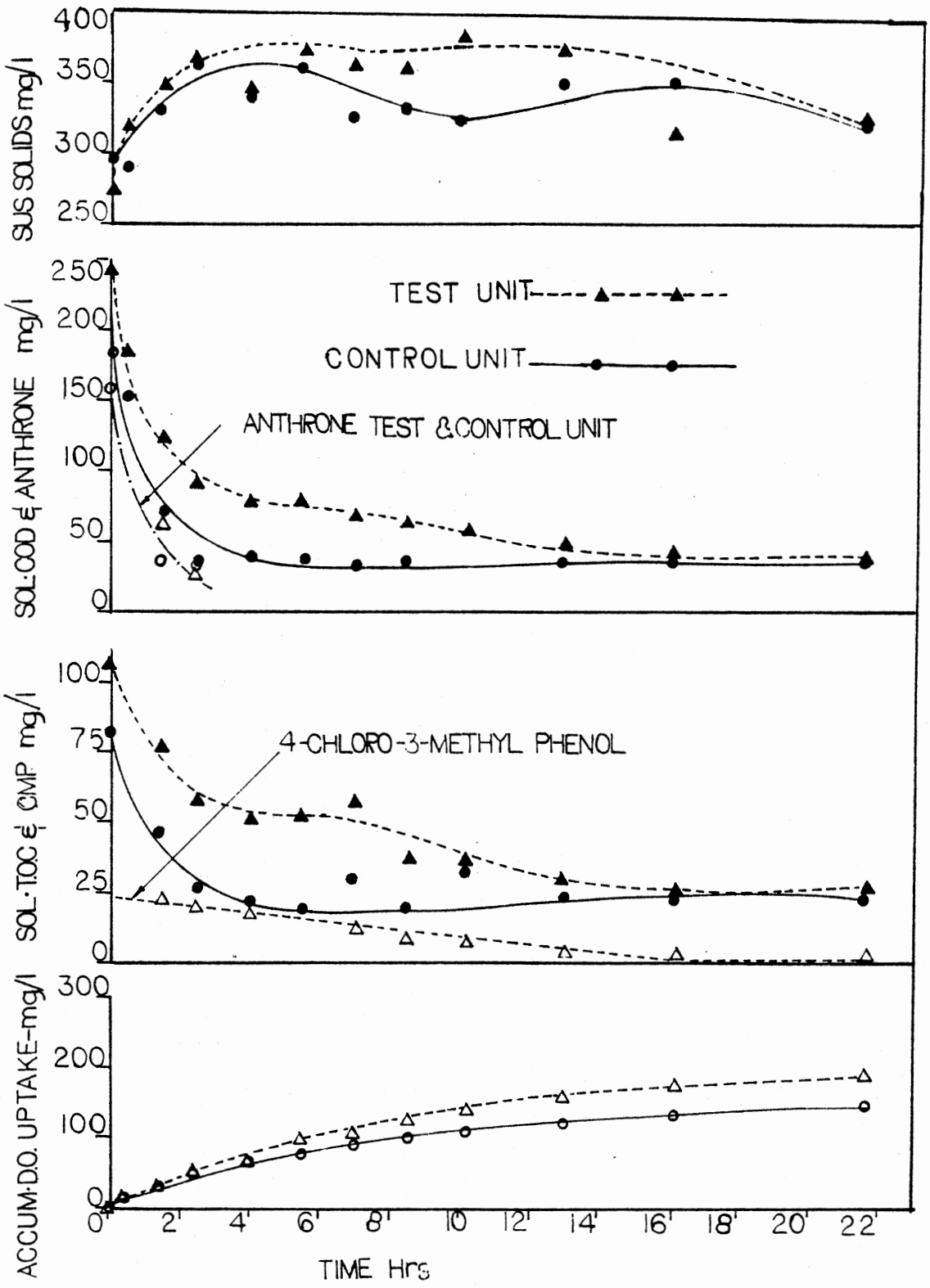


Figure 9. Substrate Removal Test; Acclimiated to 25 ppm CMP; 25°C

pattern of diphasic removal with the first stage (0-4 hours) corresponding to the control removal curve and the second phase (7-14 hours) corresponding to the CMP removal as indicated by GC analyses. Soluble anthrone reactive compounds were removed at the same rate and with the same efficiency in the control and test unit. Anthrone reactive compounds were removed in the first phase in the test unit. Dissolved oxygen uptake accumulated after 24 hours indicated that an additional 40 mg/L of dissolved oxygen was required by the test unit. This corresponds well with the fact that both units appeared to have started and finished the run with approximately the same suspended solids concentrations meaning that the additional 40 mg/L of oxygen uptake could be explained by the theoretical oxygen demand of the CMP administered.

4.2.3 50 mg/L CMP

Figure 10 shows the growth and substrate removal characteristics of the test unit after 37 days of acclimation to 50 mg/L CMP. Differences noted in growth characteristics between the control and test unit (as indicated by suspended solids measurements) were, first, that five hours were required for the test unit to reach its maximum solids concentration while only two and one half hours were required for the control and, second, test unit solids achieved a 4 percent to 5 percent greater suspended solids concentration than that of the control between five and approximately twelve hours. At the end of the run, however, both units appeared to have comparable suspended solids levels. Substrate removal in the test unit in terms of COD and TOC showed a diphasic removal compared with the one step removal of the control. Anthrone analyses indicated that the glucose was removed in the first phase while GC analyses

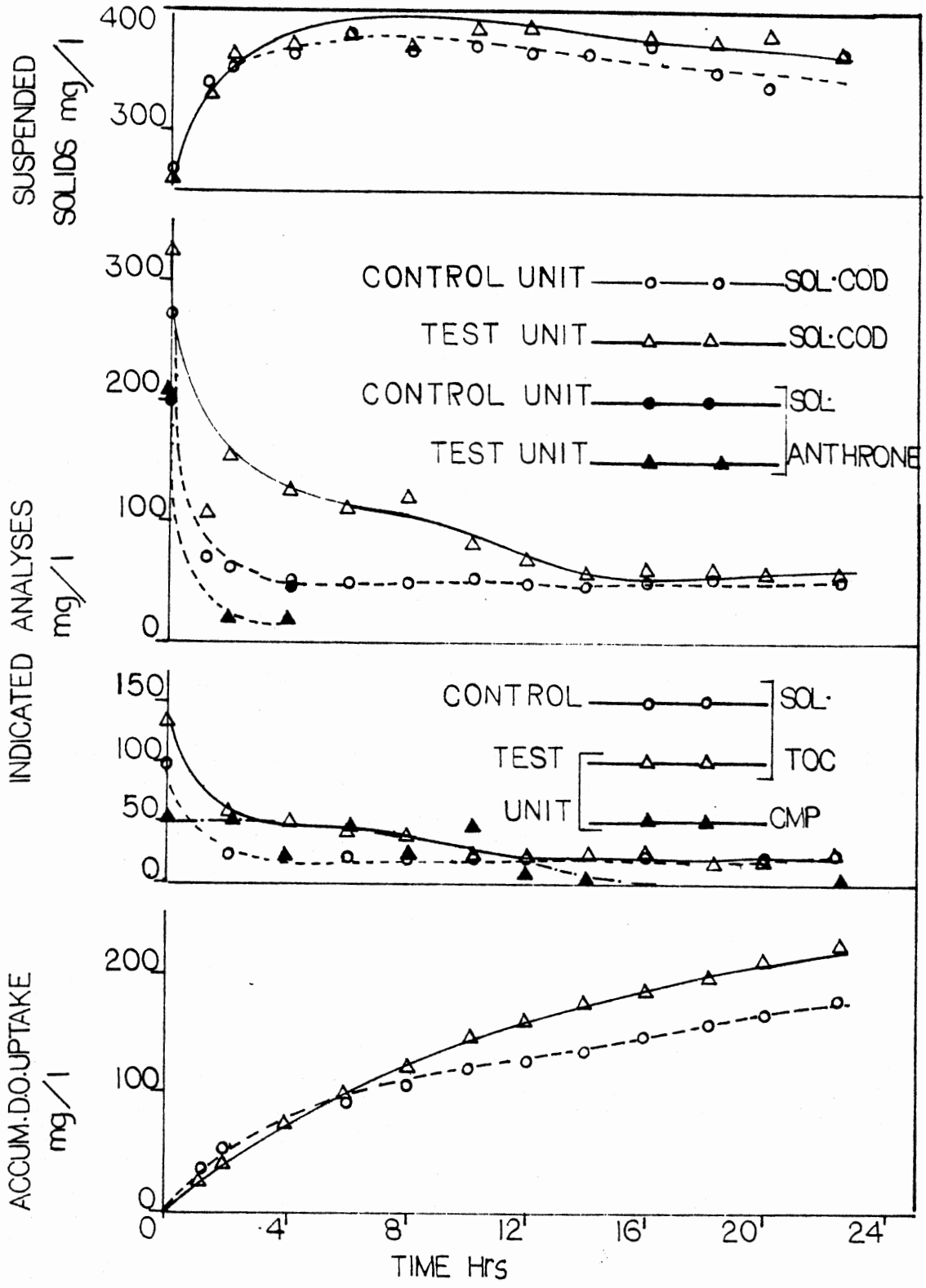


Figure 10. Substrate Removal Test; 50 ppm CMP; 19°C

confirmed that the CMP was being removed during the second phase. Based upon three data points, removal rates and efficiencies for anthrone reactive substances were the same for both the test and control units. Oxygen uptake characteristics indicated that after six to eight hours, the test unit had a higher oxygen demand relative to the control and, by the end of the run, had a cumulative oxygen uptake 49 mg/L in excess of that of the control.

4.2.4 CMP as Sole Substrate

Figure 11 presents the data collected pertaining to substrate removal, biological growth, and respiration of a batch fed biological system receiving 4-chloro-3-methyl phenol as the sole carbon substrate. During the time period from zero to approximately ten days, very little biological growth or substrate removal (beyond that attributable to air stripping) occurred. The initial increase in suspended solids concentration may have been the result of the development of an inorganic precipitate. This was suspected to be the case, since no oxygen uptake or net COD removal (beyond that due to stripping) accompanied the initial solids increase.

After approximately ten days of this lag period, biological activity was noted with increase in suspended solids, suspended protein (49% of suspended solids), and suspended carbohydrate (26% of suspended solids). At the same time, dissolved oxygen uptake rate increased while soluble COD and TOC exhibited decreasing trends.

Because there were two substrate removal mechanisms operating--namely, biological removal and air stripping--a stripping unit was operated in conjunction with the biological unit in order to be able to determine

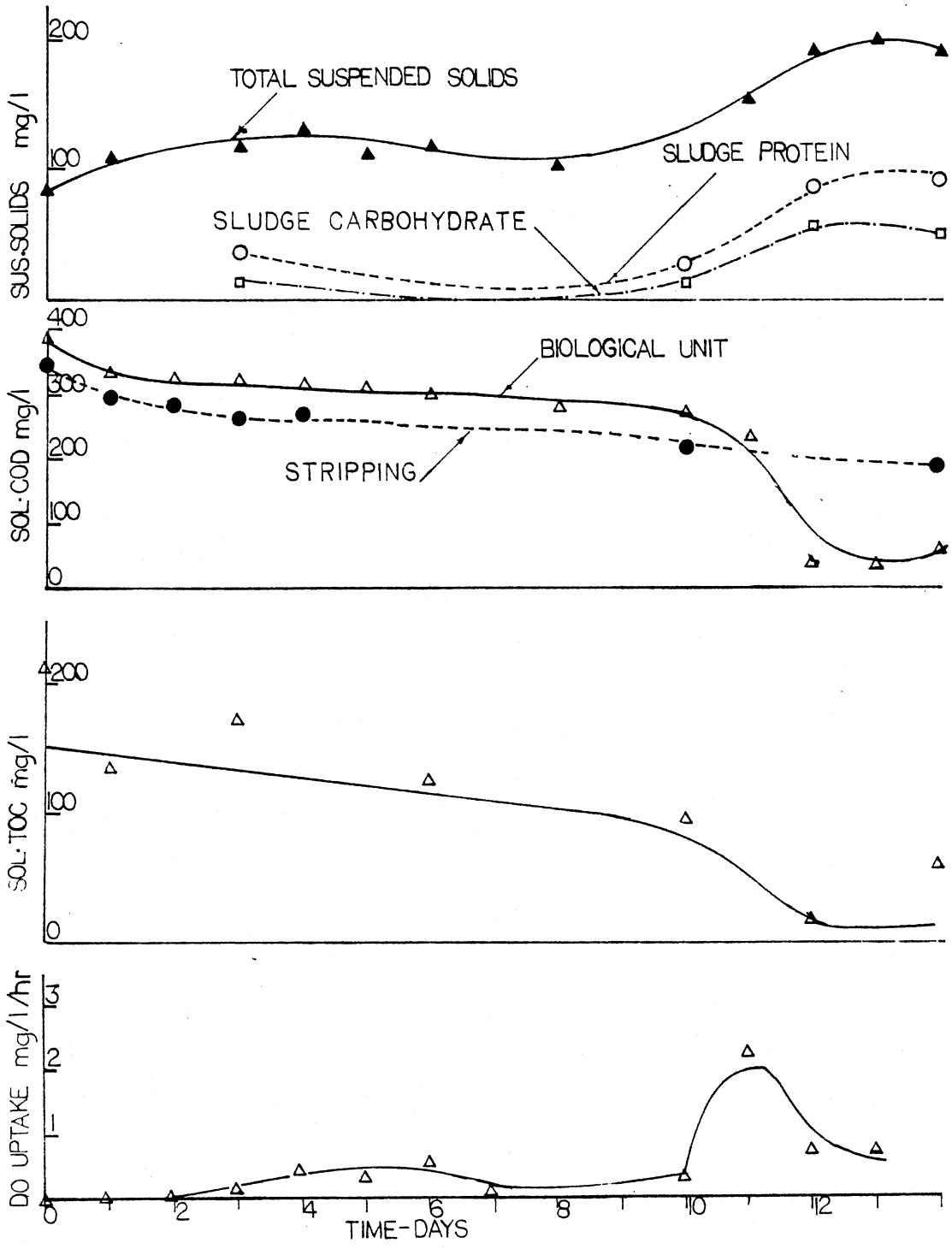


Figure 11. Batch Substrate Removal Test Utilizing CMP as the Sole Organic Carbon Source; pH = 7.7-8.3

the significance of each of the mechanisms. Under the conditions of this experiment (pH 7.7-8.3, 19°C and 1.33 L air/min/L aeration volume). CMP stripped at a constant rate of 0.31 mg COD/L/hr (0.17 mg CMP/L/hr). TOC data also exhibited a decreasing trend in the biological unit during the time in which biological activity (as measured by dissolved oxygen uptake and solids production) was low, indicating that this decrease in TOC was due to air stripping.

Overall, a COD removal efficiency of 84 percent was observed between 10 and 12 days. If we assume that the rate of air stripping remained constant during these two days, only 15 mg/L of the total 233 mg/L decrease in COD could have been removed by this mechanism. Biological uptake of CMP appeared to be the predominant removal mechanism accounting for the reduction of 94 percent of the ΔCOD between 10 and 12 days. It should also be noted that both COD and TOC data showed increases in concentrations between the twelfth and fourteenth day. Using a value of 1.42 g COD/g biological solids, a solids yield value of 50 percent was observed based upon COD.

4.3 2-Nitrophenol

4.3.1 Long-Term Data

Much of the long-term data were performed by a coworker, Whang (32), whereas this work involved a more detailed study of substrate removal characteristics utilizing 2-NP. A re-evaluation of Whang's data was made and the same statistical criteria were applied to gauge the significance of any discrepancies.

4.3.2 Control Period

Figure 12 graphically illustrates the performance of the control data and 2-NP test unit during Whang's study. A statistical analysis appears in Table VII. During the brief control period, any differences noted between soluble 24-hour COD and 24-hour suspended solids were found to be insignificant statistically (Figures 13 and 14). Biological solids yield data (Figure 15), however, indicated that possibly significant differences beyond one standard deviation from the inherent variability expected between two control units had occurred. It should be stressed, however, that the number of control points selected for comparison was less than the ten data point base period utilized to determine inherent control unit variability. Strictly speaking, then, a truly accurate statistical comparison cannot be made here.

4.3.3 5 mg/L 2-NP Dosage

Upon administration of 5 mg/L 2-NP, nearly all the variability between the control and test unit with respect to 24-hour soluble COD or suspended solids was within one standard deviation of the expected inherent variation between control units. Solids yield data indicated that there may have been a weak trend of having the test unit yield of solids greater than that of the control. It was noted by Whang (32) and verified in this subsequent work that, initially, the characteristic yellow color of 2-NP was found in the 24-hour supernatant of the test unit after the first day of feeding 5 mg/L. This "bleed-through" persisted for eight days, after which time it ceased, indicating possible metabolism of the 2-NP by the sludge. Since the amount of soluble COD imparted by the 5 mg/L 2-NP was quite small in relation to the soluble 24-hour COD of the

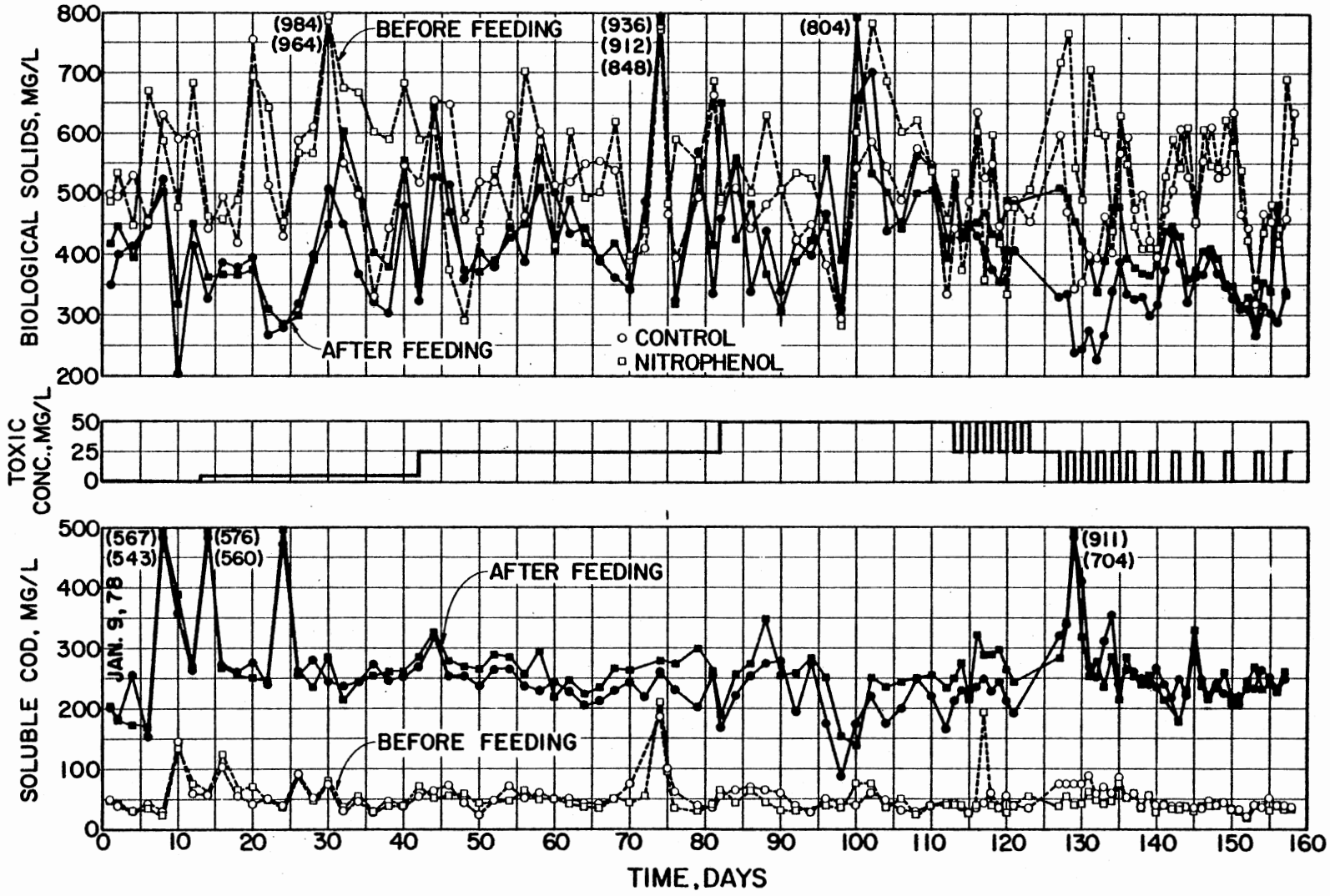


Figure 12. Long-Term Suspended Solids and Soluble Substrate Concentrations for a Batch Control Unit and a Test Unit Exposed to Increasing Concentrations of 2-NP

TABLE VII

LONG TERM STATISTICAL ANALYSIS FOR A BATCH UNIT SUBJECTED TO VARYING DOSAGES OF 2-NITROPHENOL

Operating Days	2-NP Dosage mg/L		Control Unit: Initial sol.COD mg/L	24 hr sol.COD mg/L	24 hr SS mg/L	Initial SS mg/L	Yield	pH	2-NP Test Unit: Initial sol.COD mg/L	24 hr sol.COD mg/L	24 hr SS mg/L	Initial SS mg/L	Yield	pH
1-11	0	n	7	7	7	7	7	7	7	7	7	7	7	7
		$\bar{x}$	280	56	544	394	.739	7.7	279	56	555	432	.678	7.7
		σ	134	41	65	99	.583	0.2	149	38	95	60	.519	0.2
12-25	5	n	7	6	7	7	6	5	7	7	7	7	6	5
		$\bar{x}$	334	65	522	337	.724	7.7	337	70	539	343	.818	8.1
		σ	128	26	119	52	.603	0.2	139	29	98	43	.634	0.2
26-41	5	n	8	8	7	8	7	7	8	8	7	8	7	7
		$\bar{x}$	256	47	567	395	.849	7.8	256	49	679	455	1.01	7.8
		σ	16	14	205	78	.914	0.2	24	18	133	91	.682	0.4
42-61	25	n	10	10	10	10	9	10	10	9	10	10	9	10
		$\bar{x}$	254	54	554	452	.490	7.5	274	52	511	455	.295	7.7
		σ	26	15	74	70	.254	0.3	29	7	112	82	.419	0.2
62-80	25	n	9	9	10	9	7	9	8	9	10	9	7	9
		$\bar{x}$	235	61	532	467	.669	7.7	263	47	555	470	.646	7.9
		σ	28	51	140	194	.488	0.4	24	20	115	176	.455	0.4
81-95	50	n	8	7	8	8	6	7	8	7	8	8	7	7
		$\bar{x}$	256	54	462	428	.205	7.7	265	47	526	451	.448	7.9
		σ	82	15	44	70	.207	0.2	44	14	54	111	.403	0.2
96-111	50	n	6	6	8	6	6	8	6	6	6	6	6	8
		$\bar{x}$	185	42	491	523	.185	7.8	217	50	598	531	.616	7.8
		σ	55	11	112	148	.431	0.2	45	22	169	143	.554	0.1

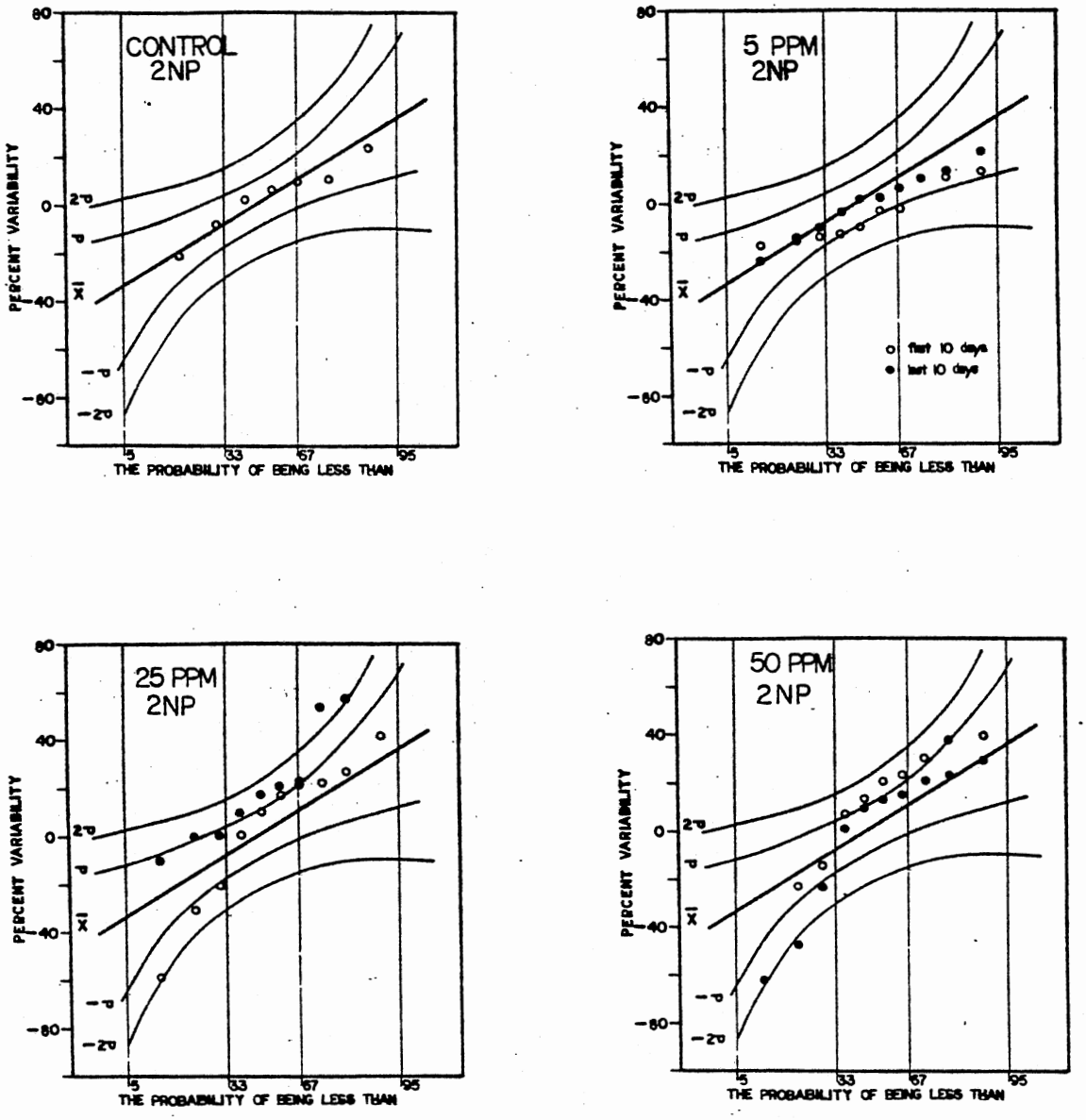


Figure 13. Analysis of Statistically Significant Effects of Various Dosages of 2-NP Upon 24-Hour Soluble COD in the Batch Unit

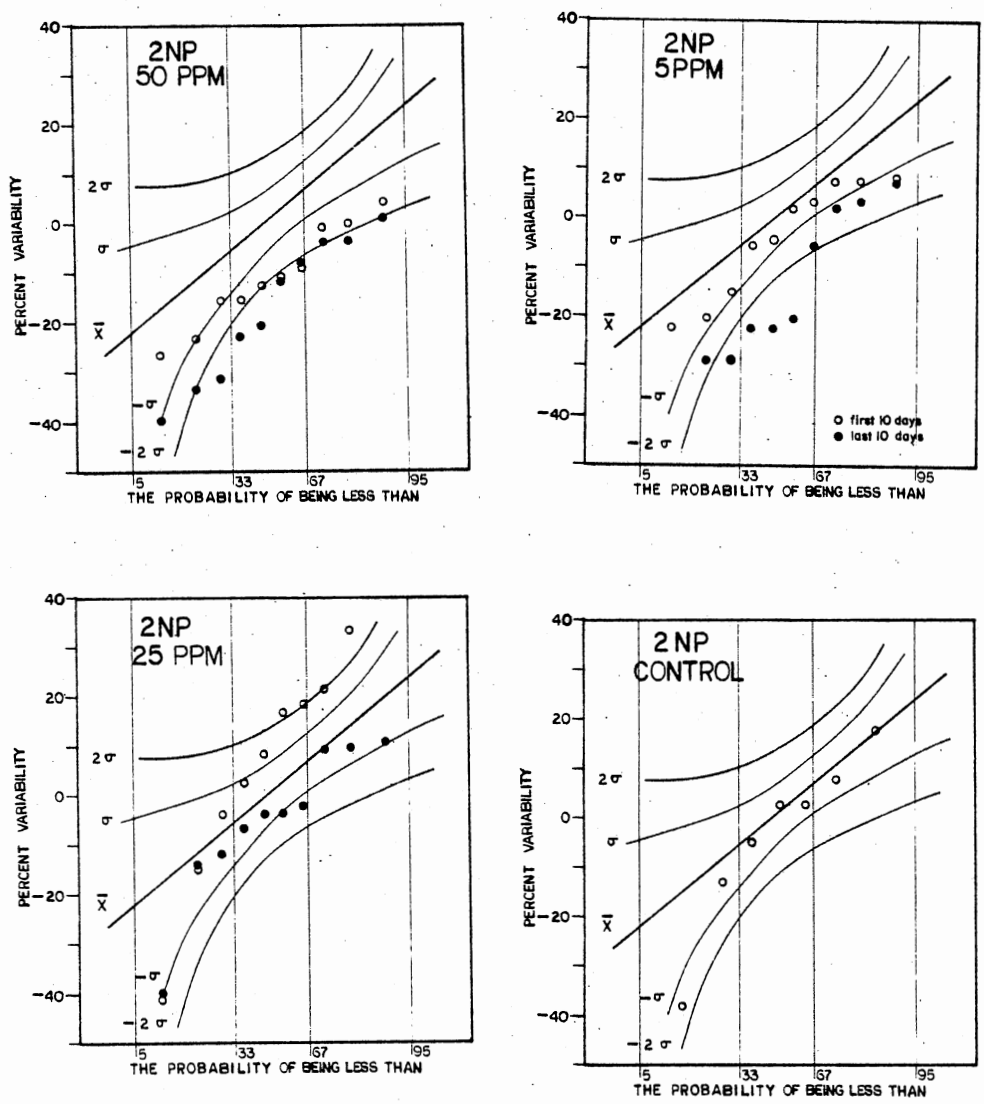


Figure 14. Analysis of Statistically Significant Effects of 2-NP Upon 24-Hour Suspended Solids in the Batch Unit

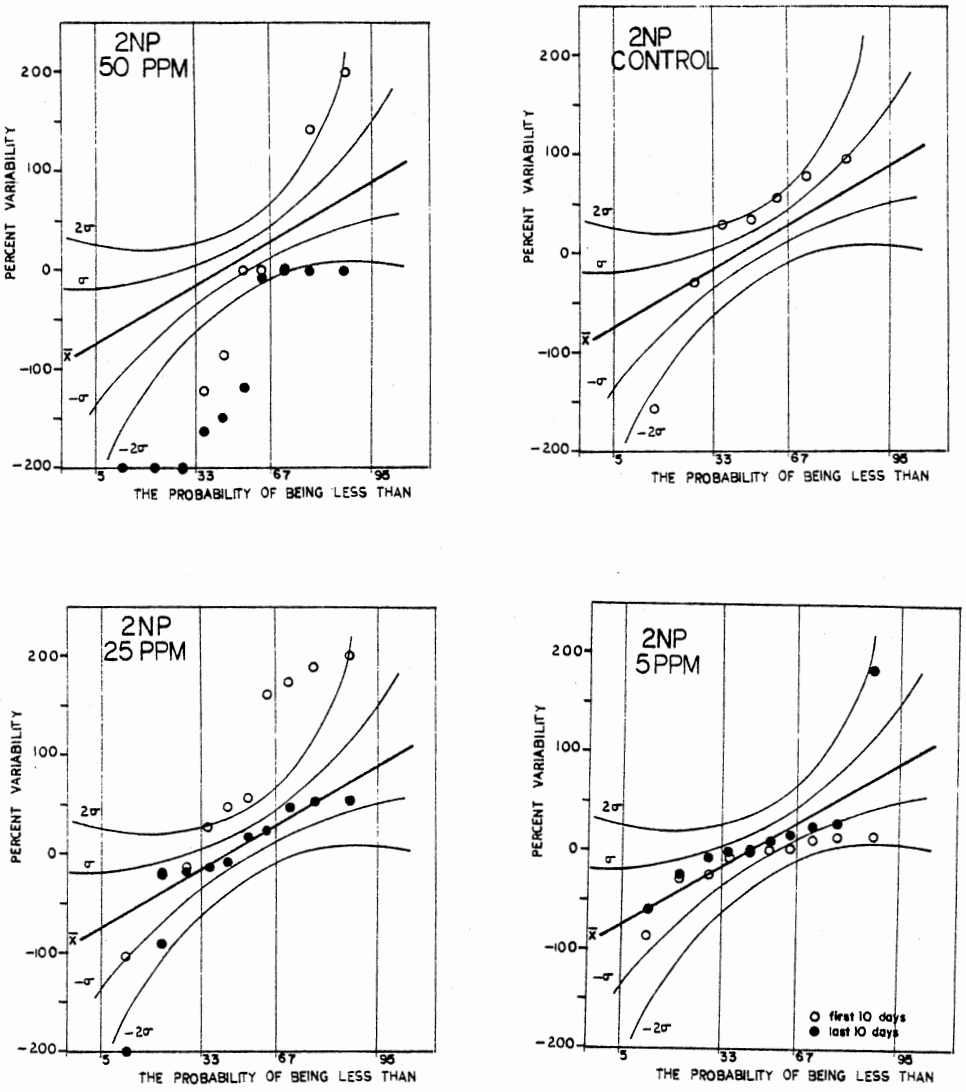


Figure 15. Analysis of Statistically Significant Effects of Various Dosages of 2-NP Upon Solids Yield in the Batch Unit

test unit, it is conceivable that it could be masked by the inherent variability of the control unit the projected on the probability plot. Careful scrutiny of the data indicates that several values of the variability concerning soluble 24-hour COD were significant to one negative standard deviation of the natural inherent variability expected. The 2-NP bleed-through may account for the statistical significance of those values. Further, during the last ten sampling days of the 5 mg/L dosage, the variations between the test and control unit with respect to soluble 24-hour COD were even less significant. Solids yield discrepancies between the two units were no longer shown to be statistically significant, either. By the variability probability plot (Figure 13), 24-hour suspended solids concentrations of the test unit were found to be significantly higher than those of the control (18% higher based on mean values).

4.3.4 25 mg/L 2-NP Dosage

During the first ten sample days, upon administration of the 25 mg/L 2-NP dosage, the most striking statistical observation would have to be the sharp slope of the probability plots constructed for 24-hour soluble COD, suspended solids, and solids yield. This phenomenon may have been the result of rather sudden changes brought about during the first ten sampling days after increasing the 2-NP dose to 25 ppm. In other words, this period may have been a transition from the system conditions during the 5 mg/L dosing period and those conditions induced by the 25 mg/L 2-NP dosage. For instance, this period represented a transition with respect to soluble 24-hour COD from the 5 mg/L period, where no statistically significant discrepancies existed between the control and test unit to the last ten sampling day period of feeding 25 mg/L 2-NP, where the test unit

appears to have a statistically lower soluble COD than the control. At the same time, this transition period represented a time when 24-hour suspended solids which had been statistically lower for the control during the 5 mg/L period eventually increased to a level where no statistically significant variability existed between the test unit and control (latter part of the 25 mg/L 2-NP dose). With respect to solids yield, the variability found between the test unit and control during the latter part of the 25 mg/L 2-NP dosing period was similar to that found during the latter part of the 5 mg/L 2-NP dosing period. Yet, the transition period of the first ten sampling days at 25 mg/L 2-NP was marked by several points, indicating that the control unit solids yield was significantly higher than that of the test unit while the rest of the points indicated that no statistically valid variability existed between the two units.

4.3.5 50 mg/L 2-NP Dosage

Soluble 24-hour COD variability between the test unit and control showed no strong trend during the first ten sampling days of the 50 mg/L 2-NP dosage. Indeed, there was virtually no evidence of "bleed-through" of the 2-NP which should have manifested itself by causing higher 24-hour soluble COD values for the test unit. The final ten sampling days during which 50 mg/L 2-NP was applied showed no statistically significant variation between control and test unit soluble 24-hour COD. Solids yield and 24-hour suspended solids concentrations did seem to be affected to a statistically significant degree during the application of 50 mg/L 2-NP. Suspended solids during the first ten sampling days were higher in the test unit and this trend was even stronger during the last ten sampling days with test unit 24-hour suspended solids concentrations 22 percent

higher than those of the control. Biological solids yield also appeared higher for the test unit to a statistically significant degree with the final ten sampling days showing the strongest trend.

4.3.6 Cyclic Dosages

Whang (32) also subjected the test unit to over one week of cyclic doses of 50 and 25 mg/L 2-NP followed by about one week of cycling from zero to 25 mg/L 2-NP. Test unit 24-hour soluble COD showed no sign of deterioration, but suspended solids levels in the unit remained higher relative to the control. The long-term tests were concluded by feeding 25 mg/L 2-NP once every third day. Again, no deterioration in test unit effluent quality resulted, even on those days which 2-NP was administered. Suspended solids concentrations of the test unit and the control did become more similar during this period, however.

4.3.7 GC Data

A summary of gas chromatograph analyses performed on the test unit can be found in Table VIII. Note that no detectable quantities of 2-NP were ever found in the 24-hour supernatant or mixed liquor samples.

4.3.8 Supernatant Suspended Solids

Table IX summarizes the limited supernatant suspended solids data compiled by Whang and myself. The data do not indicate any obvious trend concerning the effect of 2-NP on the settleability of batch-activated sludge systems.

Again, periodic microscopic examination of the two units that were operated failed to produce any major differences in flocculation,

TABLE VIII

GC ANALYSES FOR 2-NITROPHENOL

Description	Calculated Feed Conc. mg/L	Measured Feed Conc. mg/L	Measured 24-Hour Supernatant Conc. mg/L	Measured Mixed Liquor Conc. mg/L
After 18 days acclimation to 5 mg/L	3.3	3.8	<0.06	<0.06
After 25 days acclimation to 5 mg/L	3.3	3.6	<0.02	<0.02
After 38 days acclimation to 25 mg/L	16.7	23.1	<0.06	<0.04
After 32 days acclimation to 50 mg/L	33.3	37.5	<0.06	<0.04
During 25-50 mg/L cycling period	33.3	---	<0.04	<0.06
During 0-25-0 mg/L cycling period	16.7	29.9	---	<0.06

TABLE IX

2-NITROPHENOL BATCH UNIT 24-HOUR SUPERNATANT
SUSPENDED SOLIDS SETTLED FOR ONE HOUR

Description	2-NP Dosage mg/L	Control SS mg/L	Test Unit ss mg/L	Data Source
First day of dosage application	25	8	8	(32)
After 38 days acclimation to 25 mg/L	25	40	56	(32)
After 30 days acclimation to 50 mg/L	50	38	42	(32)
After 31 days acclimation to 50 mg/L	50	28	22	(32)
During 0-25 mg/L cycling period	0	62	20	(32)
After 6 days acclimation to 20 mg/L	20	21	15	Author
After about 1.5 months acclimation to 20 mg/L	20	23	41	Author

protozoan species, or numbers. Very few filamentous organisms were observed in either unit.

It should also be noted that no significant differences between control and test unit pH were observed. A statistical summary of pH determination was presented earlier in Table VII.

4.4 Substrate Removal Tests--2-NP

4.4.1 15 mg/L 2-NP

Figure 16 presents the results of control and test unit substrate removal tests during which 15 mg/L 2-NP was administered to the test unit. Suspended solids concentrations, although originating and terminating at comparable levels, showed different growth patterns in the two units. Control suspended solids reached higher levels and remained somewhat higher than test unit concentrations for at least the first 12 hours of the run. Soluble COD and TOC removal rates were quite similar for both units except that the test unit soluble COD and TOC concentrations were generally elevated by an amount corresponding to the concentration of 2-NP remaining in solution. After about 10 hours, 2-NP concentrations were below the level of detection and soluble COD and TOC for the control and test unit showed little difference. Oxygen uptake data indicated a slightly higher oxygen demand (22 mg/L higher) for the test unit. It is interesting to note that the theoretical oxygen demand of the initial 2-NP present was 21 mg/L. Final pH values obtained for both units were the same and no differences in appearance were noted between the two units. It should also be noted that a one- or two-hour lag occurred before any appreciable 2-NP was removed, but that the non-2-NP component of the waste was immediately metabolized.

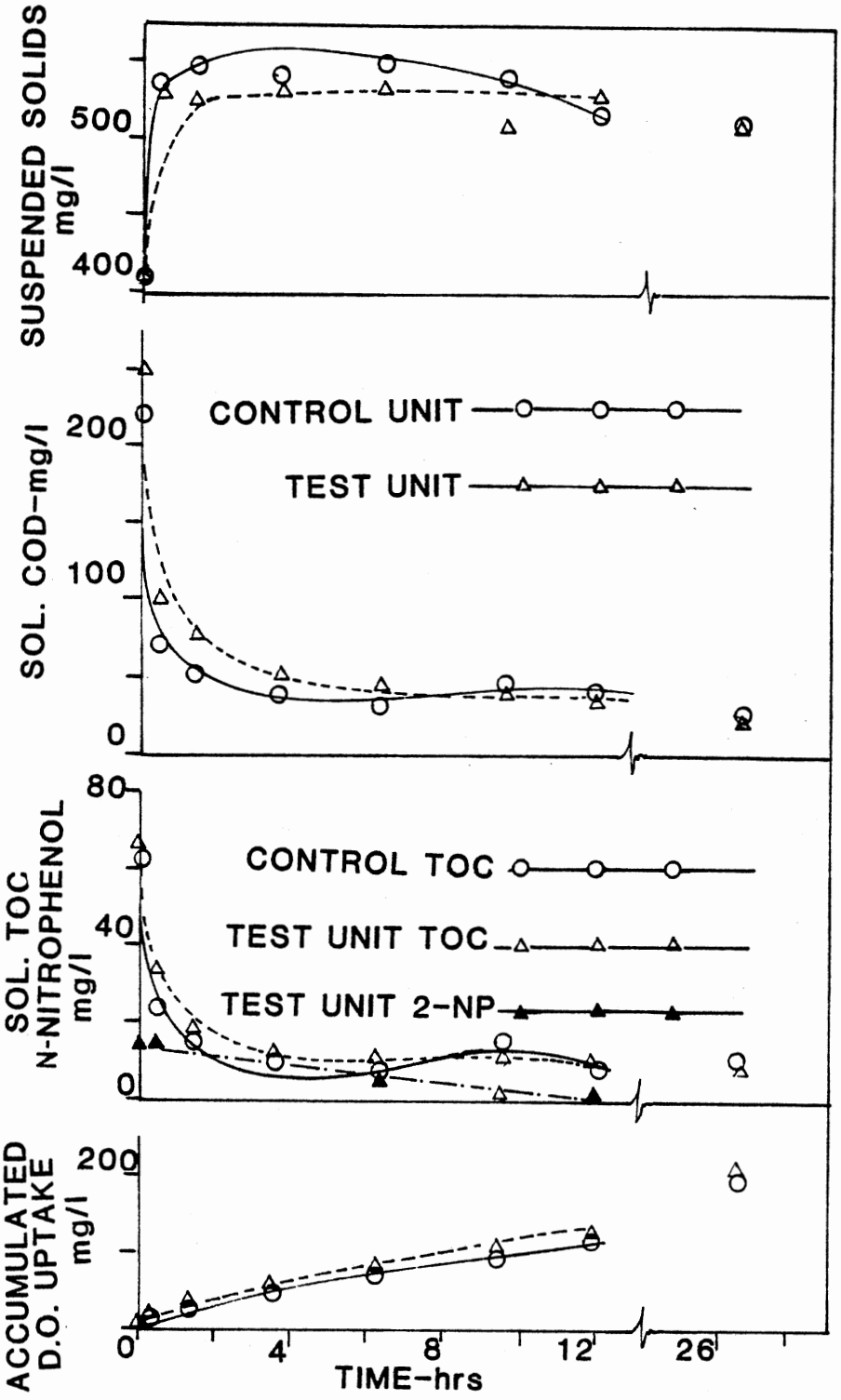


Figure 16. Substrate Removal Test; 15 ppm 2-NP; 20°C

4.4.2 20 mg/L 2-NP

Figure 17 illustrates the results of a 7-hour substrate removal test where 20 mg/L 2-NP was supplied to the test unit addition to the 200 mg/L glucose and sewage that were administered to both the control and test units. In reference to suspended solids production, the test unit, in this experiment, attained only a slightly higher concentration of suspended solids relative to the control. Soluble COD in the test unit remained higher than COD concentrations in the control unit until about 5 hours, when 2-NP analyses indicated that 2-NP concentrations had been reduced to neglibible levels. Any discrepancy between test unit and control soluble COD could generally be attributed to 2-NP remaining in the test unit. Soluble TOC data were less informative in that sometimes the control value was lower and at other times higher than that of the test unit. In addition, the final TOC of the control was found to be zero which is, in reality, quite unlikely. However, it seemed to be the trend that control soluble TOC was generally lower than test unit soluble TOC. Although there appeared to be a one-hour lag period from the time substrate was added to the test unit until 2-NP was actively removed, the other components of the substrate were immediately metabolized. Oxygen uptake data indicated that the test unit exerted a significantly higher oxygen demand than the control unit. The actual difference in oxygen demand between the two units was 20 mg/L while the theoretical oxygen demand of the initial 2-NP dosed was 28 mg/L.

4.4.3 50 mg/L 2-NP

The response of the test unit when exposed to 50 mg/L 2-NP relative to the control is shown in Figure 18. Two different growth patterns, as

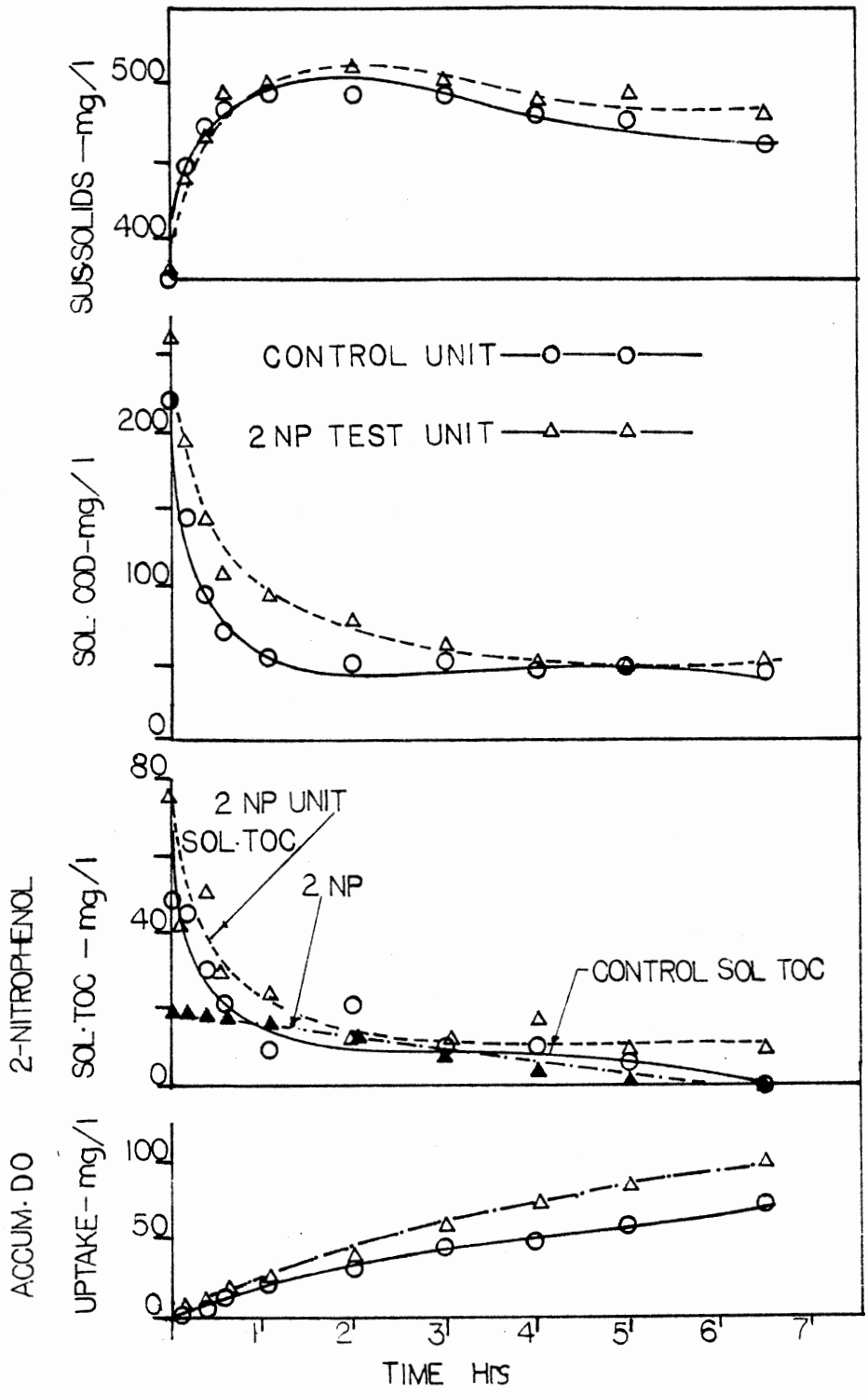


Figure 17. Substrate Removal Test; 20 ppm 2-NP; 21°C

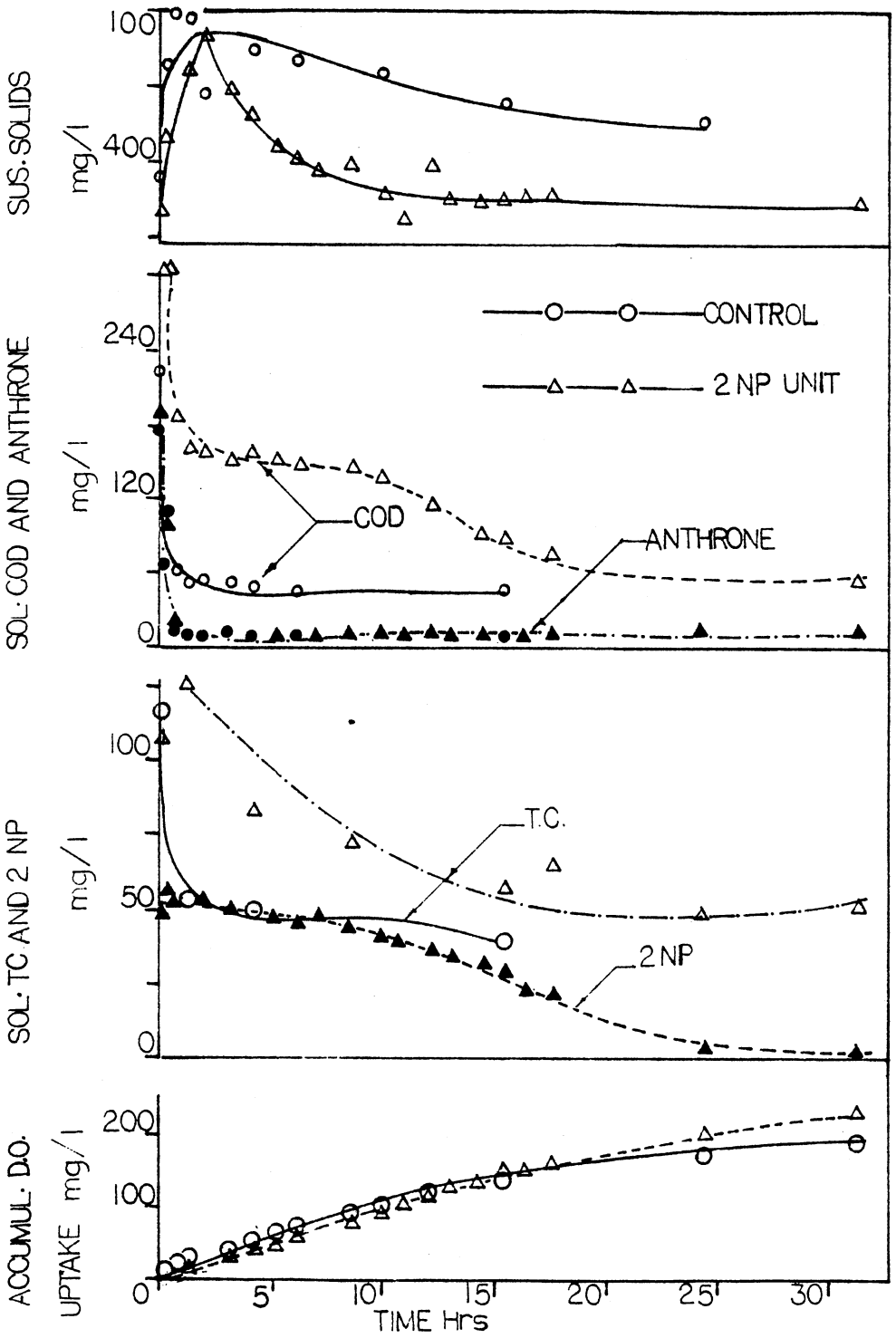


Figure 18. Substrate Removal Test; 50 ppm 2-NP

measured by suspended solids analyses, were observed. Both units achieved approximately the same maximum suspended solids concentration, but the test unit solids dropped off rapidly after reaching their maximum while the control exhibited a more gradual suspended solids decrease. After 24 hours, there was a difference of 50 mg/L suspended solids between the two units. In addition, almost no net solids increase occurred in the test unit. Substrate removal characteristics showed a pattern of the glucose component being removed rapidly (within the first hour) and the 2-NP being removed much more slowly. It was observed that there was approximately a 10-hour lag before 2-NP was removed. Anthrone analyses indicated that the removal of glucose by both units was identical with respect to both efficiency and rate. Soluble COD removal occurred in two steps in the test unit. Differences in soluble COD between the two units could be accounted for mainly by the 2-NP remaining in the test unit at any particular time. After 25 hours, test unit soluble COD levels decreased to levels found in the control. Soluble total carbon data, although more erratic, showed the same general trend as observed with the soluble COD data. Dissolved oxygen uptake was 44 mg/L higher for the test unit relative to the control after 31 hours. The theoretical oxygen demand of the initial 2-NP present was 75 mg/L. The fate of the 2-NP metabolites could not be determined, since an oxygen balance could not account for the substrate removed in either the suspended solids produced or by the oxygen consumed.

4.4.4 2-NP as Sole Substrate

Figure 19 illustrates the growth, substrate removal, and respiratory characteristics of a batch fed activated sludge unit receiving 2-NP

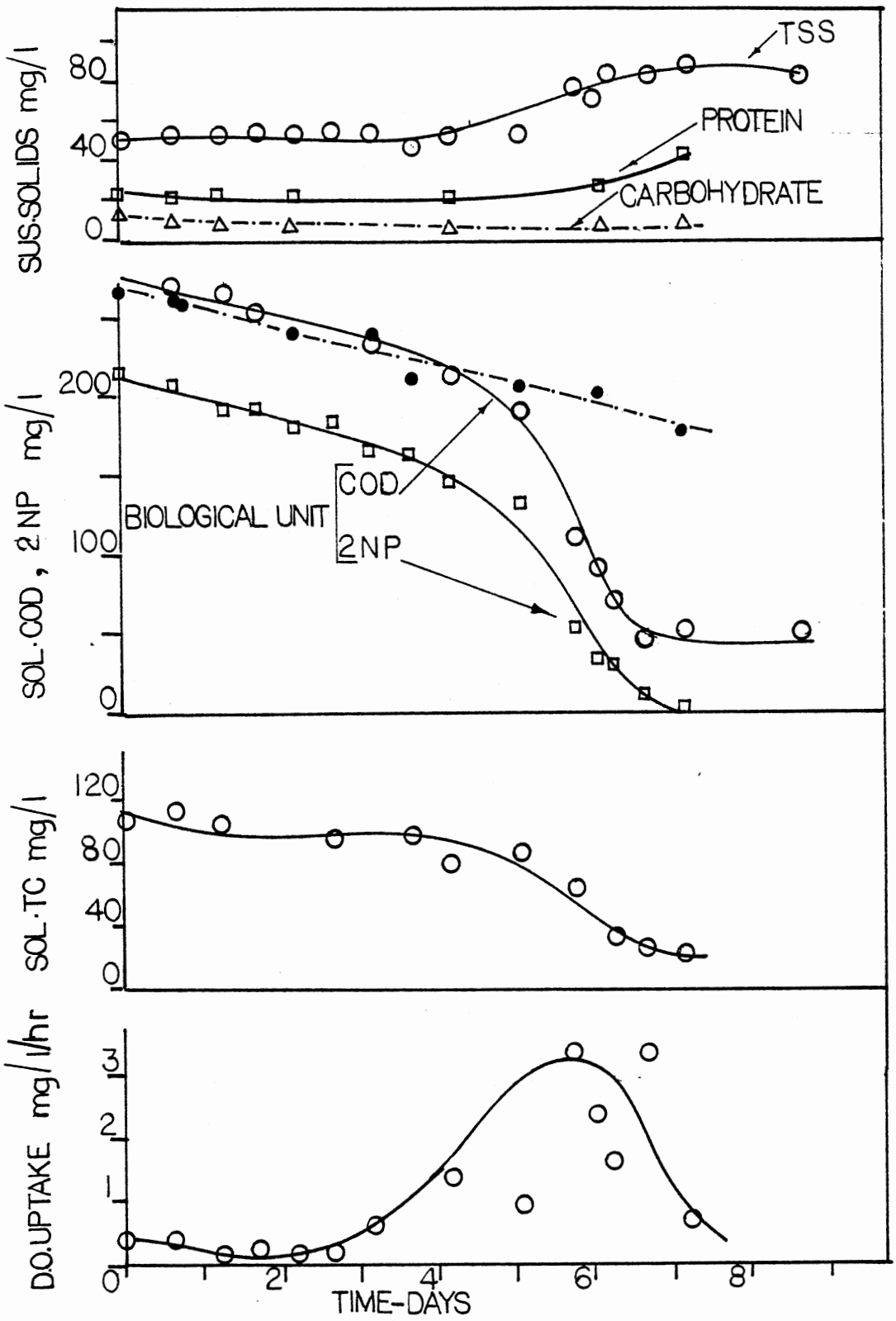


Figure 19. Batch Substrate Removal Test Utilizing 2-NP as the Sole Organic Carbon Source; pH = 8.0; Temperature = 19°C

as the sole carbon source. In addition, a parallel unit which contained no biological organisms was operated in order to measure the magnitude of air stripping. Air stripping of 2-NP under the conditions of this experiment (pH 8.0, 19°C, and an air flow rate of 1.33 L/min/L-aeration volume) was found to be 0.517 mg COD/L/hr.

In the biological unit, it can be seen that for a period of three to four days, soluble COD removal closely paralleled COD removal in the stripping unit. This, coupled with the fact that dissolved oxygen uptake rate and increase in biological solids were negligible during this same time period, suggests strongly that air stripping was the only substrate removal mechanism operating and that the first three to four days were a lag phase with respect to biological activity. In regard to biomass production, it was noted that suspended solids and protein concentrations began an increasing trend after day five and reached a maximum on day seven. The highest dissolved oxygen uptake activity occurred at this time, providing even more evidence that the 2-NP was being metabolized by the microorganisms. During this period of oxygen uptake and suspended matter accumulation, all measurements of substrate concentration (soluble COD, total carbon, and colorometric 2-NP analyses) were rapidly decreasing. It is interesting to note that suspended carbohydrate concentrations remained constant during the metabolic period, suggesting that 2-NP was not converted to carbohydrate storage products. The primary substrate removal mechanism operating between five and seven days appeared to be biological uptake, since air stripping during this period could only have accounted for a maximum 13 percent COD removal efficiency while the actual observed efficiency was 73 percent. Soluble 2-NP (colorometric) and total carbon removal efficiencies were 99 percent and 73 percent, respectively.

Assuming a value of 1.42 g COD/g of suspended solids produced, a solids yield value of 37 percent was achieved based upon COD.

CHAPTER V

DISCUSSION

Probably the most striking observation of this entire effort was the effect that 4-chloro-3-methyl phenol had upon the batch culture. It seemed as though a totally different compliment of microorganisms adapted to the CMP test unit as indicated by sludge color and test unit pH. As shown by subsequent substrate removal tests and during the long term data cyclic dosing period, the pH discrepancy between the control and test unit was not due to CMP or its metabolites since the difference in pH was noted with previously acclimated sludge even if the CMP was omitted from the feed on a particular day. Rather, the difference may have been that the microorganisms selected by the CMP feed mixture possess a sequence of metabolic pathways that led to higher pH intermediates, relative to the control, which were excreted from the cells.

It was also found that the test unit suspended solids concentrations were reduced by the 4-chloro-3-methyl phenol exposure beginning during the end of the 5 mg/L dosing period and continuing throughout the rest of the study. Since suspended solids yield values showed no evidence of reduction, it could not be concluded that CMP acted as an uncoupler of oxidative phosphorylation in the test unit. Rather, it seemed more feasible that the lower suspended solids concentrations were due to reduced settleability and loss of biomass in the supernatant subsequently withdrawn. Indeed, it was found that 24-hour supernatant suspended solids

were consistently higher in the test unit relative to the control. Furthermore, analyses of soluble carbohydrate and priority pollutant concentrations indicated that this high residual COD was not due to the initial substrates.

Substrate removal rates of the glucose and sewage component appeared to be unaffected by any CMP present. It should be pointed out that for the substrate removal tests reported here, both the test and control unit were previously acclimated to CMP. Tests were conducted pertaining to substrate removal utilizing a control unit which had not been exposed to CMP and these results are included in another report (10). Contrary to what is reported there, it is the author's judgment that substrate removal rates, at least for the glucose and sewage portion of the wastewater, for the control and test unit were quite similar. The differences between the two units could most likely be attributed to the diphasic substrate removal characteristics exhibited by the test unit, where glucose and sewage components were metabolized first and then, after a lag period, the CMP was utilized. It is contended that, in the batch units observed, glucose uptake was not inhibited by CMP and the only discrepancies noted between the test and control unit were due to residual CMP remaining in solution. This hypothesis is supported by comparson with GC analyses for the cases where the control was previously acclimated to CMP which are reported here. It could also be inferred from the data reported in Reference (10) (where the control unit had never been exposed to CMP) since the amount of COD removed from each unit after approximately one hour was quite similar even though the residual COD levels in each unit differed. This leads to another important finding which was that there appeared to be a lag between the commencement of glucose-sewage

uptake and the metabolism of the CMP. The explanation for this lag was not determined, but a phenomenon similar to the sequential substrate removal reported by other researchers (4, 9, 31) was certainly evident here.

As for the fate of CMP in the batch activated sludge system, rather conclusive evidence was obtained from the substrate removal test which utilized CMP as the sole organic carbon source. Substrate removal, oxygen uptake, and suspended protein and carbohydrate analyses demonstrated that CMP could be metabolized by microorganisms and that protein and carbohydrate could be produced as a result of this metabolic activity.

The effect of 2-nitro phenol upon the long term performance of the batch test unit was much less noticable. Sludge settleability, 24-hour pH, 24-hour soluble COD and sludge appearance were found to be nearly the same for the test and control units. In fact, it was not until administration of the 50 mg/L CMP dose that any statistically significant changes were noted in the test unit regarding 24-hour suspended solids concentrations and solids yield. Even here, the magnitude of the differences were not nearly as extreme as those observed for 4-chloro-3-methyl phenol. Furthermore, yield values for both the test and control units during the 50 mg/L 2-NP dosing period were unusually erratic with some values being negative (suspended solids reduction rather than production). It may be that some outside influence may have been responsible for these apparent significant differences rather than the 2-NP.

As with the CMP, the 2-NP appeared to have no effect on the uptake rate of the glucose component of the feed. And again, there was clear evidence of diphasic substrate removal with the glucose fraction being removed first and the 2-NP portion removed after a lag. As with the CMP, the cause of the lag is not readily apparent. It could be that the

presence of glucose prevented metabolism of the 2-NP (or CMP). However, even the substrate removal tests employing either CMP or 2-NP as the sole carbon substrate demonstrated that a lengthy lag period occurred even without the presence of glucose. Another possible explanation might be that a certain time period is required to induce the enzymes responsible for the metabolism of these two priority pollutants.

The fate of 2-NP as determined by the pure compound substrate removal test was shown to be that it was metabolized by the microbial population and some of the 2-NP was used to produce sludge protein. Unlike CMP, no increase in sludge carbohydrate concentration was observed, suggesting that carbohydrate storage products could not be produced from 2-NP.

One practical consideration concerning the fate of priority pollutants in powdered activated carbon-activated sludge wastewater water treatment systems was brought to mind when reviewing some field work performed in that area (16). When a waste water contains both biodegradable (in this case, 2-NP) and non-biodegradable priority pollutants (in this case, 2-nitro-analine), in an attempt to remove the non-biodegradable compound with the carbon, will the biodegradable compound be adsorbed and no longer be accessible to the microorganisms for metabolism. The data presented are not conclusive but this could be true and under such circumstances, the waste sludge may contain greater concentrations of these undesirable chemicals creating land filling restrictions. To meet effluent discharge and sludge disposal requirements, conventional carbon columns following biological treatment may prove to be more acceptable.

In regard to microbial toxicity, the data are not so clear. For the substrate removal tests performed where soluble carbohydrate was monitored,

there was no apparent adverse effect brought about by even the 50 mg/L dosage of either priority pollutant with respect to glucose removal rate and efficiency. However, 4-chloro-3-methyl phenol did cause a settleability problem with the test unit sludge which may have led to higher 24-hour soluble COD (intermediates) and lower suspended solids. It could be speculated that this situation was brought about by some toxic effect CMP had upon the test unit microogranisms. The lag period observed for both substrate removal tests utilizing the pure priority pollutants at high concentrations (near 250 mg/L) may have been due to a toxic effect imparted upon the test unit organisms initially but which was later overcome.

Conclusions

Table X breifly summarizes the most significant trends observed in the data collected from the long term operation of the batch units. It can be seen that 4-chloro-3-methyl phenol affected soluble 24-hour COD, 24-hour suspended solids, supernatant settleability, and 24-hour pH to a statistically significant degree during one or more dosing periods. The 2-nitrophenol had considerably less effect on the performance of the batch unit and even the two parameters indicated as being statistically different from the control are subject to question.

Substrate removal tests for both priority pollutants showed diphasic removal with the glucose-sewage component being removed first and the priority pollutant being removed last.

The rate and efficiency of glucose removal in the batch substrate removal studies did not seem to be affected by the presence of either priority pollutant.

TABLE X

SUMMARY OF THE EFFECT OF THE TWO PRIORITY POLLUTANTS
ON THE BATCH ACTIVATED SLUDGE SYSTEM

	4-Chloro-3-Methyl Phenol			2-Nitro Phenol		
	5 mg/L	25 mg/L	50 mg/L	5 mg/L	25 mg/L	50 mg/L
Soluble 24-Hour COD	0	0	+	0	0	0
24-Hour Suspended Solids	0	-	-	0	0	-
Biological Solids Yield	0	0	0	0	0	+
24-Hour Supernatant Suspended Solids	+	+ (1st 10 days)	0	No data	0	0
24-Hour pH	0	+	+	0	0	0

0 - No definite trend noted
+ - Significantly higher than control
- - Significantly lower than control

In batch substrate removal studies employing low biomass and high priority pollutant concentrations (approximately 250 mg/L) where the priority pollutant was the sole organic carbon source, long lag periods lasting several days occurred before the onset of biological uptake.

Gas chromatograph analyses indicated that the priority pollutant was removed from solution to very low levels. Some notable exceptions did occur but these took place either upon first administration of the priority pollutant or when the dosing level was increased.

Using 4-chloro-3-methyl phenol as the sole organic carbon source, it was found that increased respiratory activity occurred and that some of the substrate removed was utilized in the formation of protein and carbohydrate biomass. The same results were found for 2-nitro phenol, except that no increase in biomass carbohydrate content was observed.

Air stripping of both 4-chloro-3-methyl phenol and 2-nitro phenol was found to occur at a constant rate and was not a significant removal mechanism under the conditions of these experiments (air flow 1.33 l/min, pH 7.5-8.5, temperature 19-23°C).

BIBLIOGRAPHY

1. Alexander, M., "Nonbiodegradable and Other Recalcitrant Molecules." Biotechnology Report, Biotechnology and Bioengineering, XV, 611-647 (1973).

2. Barth, Edwin F. and Bunch, Robert L., "Biodegradation and Treatability of Specific Pollutants." U.S.E.P.A., EPA-600/9-79-034 (Oct. 1979).

3. Broeker, B. and Zahn, R., "The Performance of Activated Sludge Plants Compared with the Results of Various Bacterial Toxicity Tests--A Study with 3,5-Dichlorophenol." Water Research, 11, 165-172 (1977.

4. Chian, E.S.K. and DeWalle, F.B., "Sequential Substrate Removal In Activated Sludge Systems Fed With Naturally Occurring Wastewater." Progress In Water Technology, 7, 235-241 (1975).

5. Czekalowski, J.W. and Skarzynski, B., "The Breakdown of Phenols and Related Compounds by Bacteria." Journal of General Microbiology, 2, 231-237 (1948).

6. "Sampling and Analysis Procedures for Screening of Industrial Waste Waters for Priority Pollutants." U.S.E.P.A., Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, April (1977).

7. Feiler, Howard, "Fate of Priority Pollutants in Publically Owned Treatment Works." U.S.E.P.A., EPA-440/1-79-300, October (1979).

8. Gaudy, A.F., Jr., and Engelbrecht, R.S., "The Stripping of Volatile Compounds." Proceedings, 15th Industrial Waste Conference, Purdue University, 224-235 (1960).

9. Gaudy, A.F., Jr., Gaudy, E.T. and Komolrit, K., "Multicomponent Substrate Utilization by Natural Populations and a Pure Culture of Escherichia coli." Appl. Microbiol., 11, 157-162 (1963).

10. Gaudy, A.F., Jr., Kincannon, D.F. and Manickam, T.S., "Treatment Compatability of Municipal Waste and Biologically Hazardous Industrial Compounds." E.P.A. Grant No. R805242, presently in publication process.

11. Haller, H.D., "Degradation of Mono-substituted benzoates and Phenols by Wastewater." Journal Water Pollution Control Federation, 50, 2771-2777, Dec. (1978).

12. Heidman, J.A., Kincannon, D.F. and Gaudy, A.F. Jr., "Metabolic Response of Activated Sludge to Sodium Pentachlorophenol." Proceedings, 22nd Industrial Waste Conference, Purdue University, 661-674 (1967).

13. Irvine, R.L. and Busch, A.W., "Sequencing Batch Biological Reactors--an Overview." Journal Water Pollution Control Federation, 51, 235, February (1979).

14. Keith, Larry H. and Telliard, William A., "Priority Pollutants; I. A Perspective View." Special Report, Environmental Science and Technology, 13, April (1979).

15. Kincannon, D.F. and Gaudy, A.F. Jr., "Functional Design of Aerobic Biological Waste Water Treatment Processes." Dept. of Bioenvironmental Engineering, School of Civil Engineering, Oklahoma State University, Stillwater, Oklahoma.

16. Kincannon, D.F. and Esfandi, Ayoub, "Field Testing of the Treatability of Priority Pollutants Pharmaceutical Industry." E.P.A. Grant No. R-805242, presently in publication process.

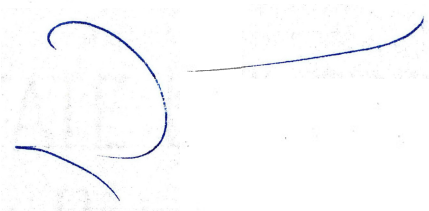
17. Patterson, James W., Brezonik, Patrick L. and Putnam, Hugh D., "Sludge Activity Parameters and Their Application to Toxicity Measurements and Activated Sludge." Proceedings, 24th Industrial Waste Conference, Purdue University, 127-155, (1969).

18. Manickam, Thillaiyambur Swaminathan, "Studies on the Effects of Combined Qualitative and Quantitative Shock Loadings on an Activated Sludge Process with Constant Concentration of Recycle Sludge." Ph.D. Thesis, Oklahoma State University, Stillwater, Oklahoma, Dec. (1978).

19. Pitter, P., "Determination of Biodegradability of Organic Substances." Water Research, 10, 231-235, (1976).

20. Proceedings of the Second Open Forum on Management of Petroleum Refinery Wastewater, Presented by the U.S.E.P.A., The American Petroleum Institute, The National Petroleum Refiners Association, The University of Tulsa, EPA-600/2-78-058, March (1978).

21. Ramanathan, M., Gaudy, A.F. and Cook, E.E., "Selected Analytical Methods for Research in Water Pollution Control." M-2 Manual, Dept. of Bioenvironmental Engineering, Oklahoma State University, August (1968).



22. Raymond, D.G.M. and Alexander, M., "Microbial Metabolism and Cometabolism of Nitrophenols." Pesticide Biochemistry and Physiology, 1, 123-130 (1971).

23. Registry of Toxic Effects of Chemical Substances. National Institute for Occupational Safety and Health (1975).

24. Shackelford, W.M. and Keith, L.H., "Frequency of Organic Compounds Identified in Water." EPA-600/476-062.

25. Simpson, J.R. and Evans, W.C., "The Metabolism of Nitrophenols by Certain Bacteria." Biochemical Journal, 55, XXIV (1953).

26. Standard Methods for the Examination of Water and Wastewater, 14th Edition. Published jointly by the American Public Health Association, American Water Works Association, Water Pollution Control Federation (1976).

27. Strier, Murray P., "Treatability of 65 Chemicals; Part A: Biochemical Oxidation of Organic Compounds." Correspondence from the Office of Quality Review, U.S.E.P.A., Washington, D.C.

28. Strier, Murray P., "Estimated Theoretical Treatability of Organic Priority Pollutants; Part E: The Relationships with Solubility Parameters." U.S.E.P.A. 440/1-79/100, May (1979).

29. Strier, Murray P., "Treatability of Organic Priority Pollutants; Part C: Their Estimated Treated Effluent Concentration--A Molecular Engineering Approach." Correspondence from the Office of Quality Review, U.S.E.P.A, Washington, D.C., 11 July (1978).

30. Strier, Murray P., "Pollutant Treatability: A Molecular Engineering Approach." Environmental Science and Technology, 14, 1, 28-31, January (1980).

31. Su, Joseph Jean-Jeag, "Studies on the Interactions of Multicomponent Carbon Sources in Discontinuous and Continuous Flow Activated Sludge Systems." Ph.D. Thesis, Oklahoma State University, Stillwater, Oklahoma, July (1970).

32. Whang, G.D., "The Effects of Organic Toxic Compounds on the Performance of a Batch Activated Sludge Process." M.S. Thesis, Oklahoma State University, Stillwater, Oklahoma, July (1979).

VITA

David Edward McCartney

Candidate for the Degree of

Master of Science

Thesis: A STUDY OF TWO PRIORITY POLLUTANTS, 4-CHLORO-3-METHYL PHENOL AND 2-NITROPHENOL, CONCERNING THEIR FATE AND EFFECT UPON SYSTEM PERFORMANCE IN THE BATCH ACTIVATED SLUDGE PROCESS

Major Field: Bioenvironmental Engineering

Biographical:

Personal Data: Born in Upper Darby, Pennsylvania, September 6, 1952, the son of Clarence E. and Dorothy R. McCartney.

Education: Graduated from Marple-Newtown High School, Newtown Square, Pennsylvania, in June, 1970; received the Bachelor of Arts degree in Biological Science from East Stroudsburg State College, East STroudsburg, Pennsylvania, in May, 1974; completed the requirements for the Master of Science degree in Bioenvironmental Engineering at Oklahoma State University in May, 1981.

Professional Experience: Assistant Project Scientist, Roy F. Weston, Inc., Environmental Consultants and Designers, 1974-1978; Graduate Research Assistant, School of Civil Engineering, Oklahoma State University, 1978-1981.

Membership in Professional Societies: Water Pollution Control Federation; American Water Works Association.